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conducted following oral and inhalation exposure to PCE, and from inhalation exposure to TRI in rats and mice. Neurobehavioral depression was compared with uptake of PCE and TRI in the brain and blood of rats, and with TRI in mice. The regional brain distribution of TRI was evaluated in mice and rats following inhalation exposure. This data was compared to the regional brain distribution of cyclic GMP resulting from TRI inhalation. The toxicokinetics of inhaled TCE was also evaluated in different brain regions in rats, and compared to the various isoforms of glutathione s-transferase in the brain. The direct measurements of halocarbon concentrations in exhaled breath, blood and tissues have provided an extensive data base that has been used to formulate and validate the physiologically-based pharmacokinetic (PBPK) models for exposure to halocarbons. The partition coefficients for developing PBPK models for rats and dogs were determined from detailed tissue determinations in these species. Using the direct measurements data, the ability of the PBPK models to generate accurate predictions of halocarbon concentrations were evaluated in blood, exhaled breath, and seven tissues.

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Interspecies Extrapolations of Halocarbon Respiratory and Tissue Kinetics: Applications to Predicting Toxicity in Different Species

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Dr. Cham E. Dallas, Principal Investigator

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**Interspecies Extrapolations of Halocarbon Respiratory and Tissue Kinetics:
Applications to Predicting Toxicity in Different Species**

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TECHNICAL SUMMARY

A series of experiments have been conducted to provide a pharmacokinetic data base for interspecies comparisons and for formulation and validation of physiologically-based pharmacokinetic models. The basic experimental design has involved evaluating the toxicokinetics of halocarbons in different species, including mice, rats, and dogs. Perchloroethylene (PCE), tetrachloroethane (TET), trichloroethylene (TCE), and trichloroethane (TRI) have been employed as test chemicals, in order to evaluate the relative importance of the physicochemical property of volatility on the kinetics and toxicity of halocarbons. In order to determine the dose received in target organs and other tissues, serial samples of brain, liver, kidney, lung, heart, skeletal muscle, and adipose tissue have been taken and analyzed for halocarbon content after administration of PCE, TET, and TRI in rats, TRI and TCE in mice and PCE and TET in dogs. For neurobehavioral studies, an operant testing system and rotord system have been employed for monitoring the central nervous system effects of halocarbons. Neurobehavioral studies have been conducted following oral and inhalation exposure to PCE, and from inhalation exposure to TRI in rats and mice. Neurobehavioral depression was compared with uptake of PCE and TRI in the brain and blood of rats, and with TRI in mice. The regional brain distribution of TRI was evaluated in mice and rats following inhalation exposure. This data was compared to the regional brain distribution of cyclic GMP resulting from TRI inhalation. The toxicokinetics of inhaled TCE was also evaluated in different brain regions in rats, and compared to the various isoforms of glutathione s-transferase in the brain. The direct measurements of halocarbon concentrations of exhaled breath, blood and tissues have provided an extensive data base that has been used to formulate and validate the physiologically-based pharmacokinetic (PBPK) models for exposure to halocarbons. The partition coefficients for developing PBPK models for rats and dogs were determined from

detailed tissue determinations in these species. Using the direct measurements data, the ability of the PBPK models to generate accurate predictions of halocarbon concentrations were evaluated in blood, exhaled breath, and seven tissues.

I. OVERALL OBJECTIVE AND SPECIFIC AIMS

The overall objective of this project is to investigate the scientific basis for the interspecies extrapolation of pharmacokinetic and neurobehavioral toxicity data. Direct measurements of the respiratory elimination and tissue concentrations of halocarbons over time in two species will be used to formulate and validate physiologically-based pharmacokinetic models for halocarbon exposure. These models will be used for: (a) prediction of the time-course of the respiratory elimination and target organ levels of halocarbons; (b) interspecies extrapolations (i.e., scale-up from smaller to larger laboratory animals and ultimately to man). A combined physiological pharmacokinetic-toxicodynamic model for halocarbon exposure will subsequently be developed and evaluated for its ability to predict neurobehavioral effects under specified exposure conditions.

SPECIFIC AIMS in the proposed studies are to:

- 1) Determine the respiratory elimination of physicochemically disparate volatile organic compounds (VOCs) in two animal species. Rats and dogs will be administered selected halocarbons by inhalation and oral exposure. Concentrations of expired parent compounds will be monitored in the exhaled breath for appropriate periods during and following exposure. Together with monitoring of the respiratory volumes of the test animals, this data will enable calculation of the cumulative uptake and elimination of the halocarbons. Data from both species will be compared to existing data sets for respiratory elimination in humans.
- 2) Delineate the tissue disposition of inhaled halocarbons in two animal species. Rats and dogs will be exposed to halocarbons by inhalation. Concentrations of the parent compound in brain, liver, heart, lung, kidney, skeletal muscle, and adipose tissue will be measured over time, in order to provide an assessment of the actual target organ dose for correlation with neurobehavioral toxicity and for development and validation of physiologically-based pharmacokinetic and toxicodynamic models.
- 3) Validate physiologically-based pharmacokinetic (PBPK) models for predicting the tissue pharmacokinetics of halocarbons in two animal species. PBPK models that have been initially developed in rats in our previous studies, with blood and expired air data for inhalation exposures and with tissue data for intraarterial and oral exposures, will be further validated for accuracy in interspecies extrapolations. Direct measurements of exhaled breath and tissue concentrations and associated parameters in rats and dogs in the initial two phases of the presently proposed project will be used to further test the accuracy of our PBPK models. Models will be developed each for the rat and the dog. The observed animal data and pharmacokinetic parameters will be used to formulate allometric relationships which can then be used to predict human disposition of halocarbons.
- 4) Correlate the neurobehavioral toxicity of inhaled VOCs in two species with the target organ concentration. Rats and dogs will be exposed to selected halocarbons at defined inhaled concentrations and lengths of exposure. Neurobehavioral tests for operant performance will be performed periodically during and after exposures. The magnitude of central nervous system (CNS) effects of each solvent will be correlated with the

target organ (i.e., brain) halocarbon concentration, as determined in (2), at each time-point. Thereby, it will be possible to determine whether equivalent target organ doses in the rats and dogs elicit CNS effects of comparable magnitude in each species.

- 5) Develop and validate toxicodynamic models for inhaled halocarbons. Brain halocarbon concentrations will be correlated with the magnitude of neurobehavioral toxicity in an appropriate equation. These relationships will be used in conjunction with the PBPK model developed in (3). The CNS effects observed in (4) will be compared to predicted effects to assess the validity of the model in the two species tested. Validated models may allow the prediction of CNS effects over time of exposure using: a) extrapolations from pharmacokinetic data; b) simulations in the absence of experimental data.

II. FUNDAMENTAL HYPOTHESIS TESTING

A very important question faced by scientists and administrators conducting risk assessments is the relevance of toxic effects seen in animals to anticipated adverse effects in humans. Pharmacokinetic studies are playing an increasingly important role in species to species extrapolations in toxicology. Blood concentration over time, as a measure of bioavailability, is routinely accepted as an index of the level of chemical in the entire system, and therefore a representative parameter of toxic effects. This assumption can be misleading, in that blood concentrations may not be reflective of the concentration of chemical or active metabolite at the local site of effect in a target organ or tissue. It is now recognized that chemical toxicity is a dynamic process, in which the degree and duration of toxic effect in each tissue is dependent upon systemic absorption, tissue distribution, interactions with cellular components, and clearance from the tissue and body by metabolism and excretion. Estimation of the risk of toxic injury from pharmacokinetic data is based on the assumption that the intensity of the response from a given dose is dependent upon the magnitude of the dose received by a target tissue. A related assumption can be stated in the form of a HYPOTHESIS:

THE DOSE RECEIVED IN A PARTICULAR TARGET TISSUE IN ONE SPECIES WILL HAVE THE SAME DEGREE OF EFFECT AS AN EQUIVALENT TARGET TISSUE DOSE IN A SECOND SPECIES.

There are surprisingly few scientific data that are applicable to this basic assumption, although it is a very important premise in interspecies extrapolations in toxicology. If valid, it is a logical basis on which to evaluate the suitability of different species as predictors of toxicity in humans (i.e., the species in which target organ deposition is most similar to man would likely be an appropriate animal model for toxicity testing).

A series of parallel studies in different animal species is therefore being employed to test the foregoing hypothesis. The rat and dog have been subjected to equivalent exposure to halocarbons. Similarities and differences in respiratory elimination, tissue disposition, and toxicity between the species are being determined. Neurobehavioral alterations will be used as a toxic end-point in the currently proposed work, since: a) OSSA and EPA commonly use neurobehavioral effects as the basis for deriving standards for exposure to VOCs; b) central

nervous system (CNS) depression is caused by and can be directly correlated with the concentration of parent compound in the CNS (Bruckner and Peterson, 1981).

III. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL DEVELOPMENT FOR RESPIRATORY ELIMINATION AND SYSTEMIC UPTAKE OF TCE IN RATS

A paper has been published during the first year of this project on the pharmacokinetics and PBPK model validation for inhaled TCE. The reprint is included as Section A of the Appendix, and the reference is as follows:

Dallas, C.E., Gallo, J.M., Ramanathan, R., Muralidhara, S., and Bruckner, J.V. "Physiological pharmacokinetic modeling of inhaled trichloroethylene in rats." *Toxicology and Applied Pharmacology* 110: 303-314 (1992).

The pharmacokinetics of trichloroethylene (TCE) was characterized during and following inhalation exposures of male Sprague-Dawley rats. The blood and exhaled breath TCE time-course data were used to formulate and assess the accuracy of predictions of a physiologically based pharmacokinetic (PB-PK) model for TCE inhalation. Fifty or 500 ppm of TCE was inhaled by unanesthetized rats of 325-375 g for 2 hr through a miniaturized one-way breathing valve. Repetitive samples of the inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently during and for 3 hr following the exposures and analyzed for TCE by headspace gas chromatography. Respiratory rates and volumes were continuously monitored and used in conjunction with the pharmacokinetic data to delineate uptake and elimination profiles.

TCE exhaled breath levels were found to have increased rapidly after the initiation of exposure to near steady-state within approximately 20-30 min and were then directly proportional to the exposure concentration. Uptake of TCE in the blood was also rapid, but blood levels continued to increase progressively over the course of the inhalation exposure at both dose levels. Arterial TCE concentrations were not proportional to the inhalation concentration, with levels for the 500 ppm group from 25-30 times greater than in 50 ppm-exposed rats during the second hour of the exposure. Percent uptake rapidly thereafter until equilibrium was established by 1 hr, after which % uptake of the inhaled dose was approximately 69-72% for both exposure groups. Total cumulative uptake of 50 to 500 ppm TRI over the 2-hr inhalation exposures was determined to be 8.4 and 73.3 mg/kg bw, respectively. The direct measurements of TCE in the blood and exhaled breath were utilized in the validation of a physiological pharmacokinetic model of the prediction of the pharmacokinetics of inhaled TCE. Results from this study indicate that metabolism of TCE is saturable between 50 and 500 ppm exposure in rats, resulting in disproportionately higher blood levels above the saturation point. At doses below this metabolism saturation point in rats, blood and exhaled breath levels of TCE in rats were very similar to values previously published for TCE inhalation exposures in humans. The PB-PK model was characterized as blood flow-limited with TCE eliminated unchanged in the exhaled breath and by saturable liver metabolism. The uptake and elimination profiles were accurately simulated by the PB-PK model for both the 50 and 500 ppm TCE exposure levels. Such a model may be quite useful in risk assessments in predicting internal (i.e., systemically absorbed) doses of TCE and other volatile organics under a variety of exposure scenarios.

IV. ANALYTICAL DETERMINATIONS OF HALOETHANES AND HALOETHENES IN TISSUES

A paper has been published in a scientific journal on the method that was developed for tissue determinations of aliphatic halocarbons. A copy of the published paper is included as section B of the appendix, and the reference is as follows:

Chen, X.M., Dallas, C.E., Muralidhara, S., Srivatsan, V., and Bruckner, J.V. "Analyses of volatile C₂ haloethanes and haloethenes in tissues: sample preparation and extraction." *Journal of Chromatography* 612: 199-208 (1993).

Characterization of the systemic uptake, distribution and elimination of volatile organic compounds (VOCs) requires reliable analytical techniques for measuring the concentration of the chemicals in different tissues of the body. An extraction procedure was developed which minimized loss of the readily volatilizable compounds, so that they could subsequently be quantified by headspace gas chromatography. The procedure was evaluated using four C₂ halocarbons [i.e., perchloroethylene (PER), 1,1,1-trichloroethane (TRI), 1,1,2,2-tetrachloroethane (TET), and 1,1,2-trichloroethylene (TCE}] of varying physicochemical properties. Portions of 0.5 to 1 g of liver, kidney, brain, heart, lung, skeletal muscle, fat and blood from rats were spiked with PER to yield a theoretical concentration of 4 µg/g tissue. Two homogenization procedures were evaluated: (a) tissues were homogenized in saline, followed by isoctane extraction; and (b) tissues were homogenized in isoctane and saline (4:1, v:v). the latter approach resulted in a significantly higher percent recovery of PER from most tissues. Neither homogenization nor the presence of saline affected PER standards prepared in isoctane. It was observed that the volume of the aliquot of isoctane taken for PER analysis was important, in that aliquots > 25µl could not be used. PER concentrations were determined in tissues of rats following *in vivo* (i.e., intraarterial administration) of the halocarbon using the latter (i.e., isoctane) homogenization approach. This approach was also employed to examine the efficiency of recovery of PER, TET, TRI, and TCE from seven tissues and from blood. Percent recoveries of each of the four halocarbons ranged from 73-104% for the seven spiked tissues. The recoveries did not appear to be tissue-dependent, despite differences in homogenization time required for different tissues. Recovery, however, did vary somewhat with test chemical, with the least volatile, most lipophilic compounds exhibiting the highest recovery.

Extensive analyses in two species of rats and dogs have been conducted to determine the percent recovery for both PCE and TET in the following tissues: brain, liver, kidney, lung, fat, heart, and muscle. In order to know the variation in percent recovery between species, PCE and TET chemicals were chosen to measure the percent recovery in both rats and dogs. For rats, four different concentrations 0.1, 1, 5 and 20 mg/ml, six groups for percent recovery of dog tissue 0.1, 0.5, 1, 5, 10 and 20 mg/ml were selected. In each group, there were 6 samples for each kind of tissue. Animals were anesthetized with ether. One ml blood samples were withdrawn by closed cardiac puncture. Approximately 1 gram of rat tissue were removed and placed on ice. In case of dog tissue, a dog was sacrificed and all recoverable tissue types were harvested and divided into small pieces which were approximately equal to 1 gram. PCE and TET at concentrations of 0.1, 1, 5, and 20 mg/ml for 4 groups of rats. 0.1, 0.5, 1, 5, 10 and 20 mg/ml for 6 groups of dogs were carefully injected into each tissue by using a Hamilton microsyringe. After injecting, the tissues were

immediately transferred into 2 ml of chilled saline and 8 ml of chilled isoctane. The samples were maintained on ice even during homogenization. A polytron tissumizer was used to homogenize the tissue samples. Samples were extracted with 8 ml of isoctane and vortexed for 30 seconds. Samples were then centrifuged at 1800 G for 5 min at 4°C in a Sorvall RC 2-B centrifuge. Twenty ul of the organic phase was withdrawn with an Eppendorf pipet and transferred to 20 ml headspace vials (Perkin-Elmer Model 8500).

Standards were made and assayed by diluting a calculated amount of pure test chemical in the appropriate solvent. The column used was 8' x 1/8" stainless-steel column packed with 3 to OV-17 on chromasorb W (100-120 mesh) operating temperatures were: injection port 200°C; electron capture detector 360°C; column 110°C for PCE, 140°C for TET; and headspace control unit 90°C.

Efficiency of recovery from rat and dog tissues for PCE and TET ranged from 87.78% to 97.8% (Table B-1.2). Low standard deviation ranging from 1.44 to 8.85 were obtained in 4 or 6 groups of rat and dog tissues. There was no big difference between the lowest concentration 0.1 mg/ml and the highest 20 mg/ml. In the two compounds and the two species of animals. Comparison of different kind of tissues of rat and dog indicated that the percent recovery was very consistent for PCE, for TET kidney, fat and muscle of rat tissue recovery were slight lower than dog's. Between PCE and TET, recoveries of PCE were a little higher than TET. In summary, efficiency of recovery at various concentrations, for both chemicals and the animals were very consistent and reproducible.

V. FURTHER DEVELOPMENT OF EXTRACTION EFFICIENCY IN TISSUE HALOCARBON MEASUREMENTS

A review of the literature revealed a paucity of methods for analysis of VOCs in organs. Currently available method(s) for measuring VOCs in tissues typically involve homogenization which invariably leads to loss of these volatile compounds due to mechanical agitation and heat buildup. A new method was developed using potassium hydroxide (KOH) solution as a dissolution medium. This approach appears to improve the overall efficiency and percent recovery of VOCs over methods currently in use.

These results were presented at the most recent meeting of the Society of Toxicology. The reference for the abstract for this presentation is:

Muralidhara, S., Srivatsan, V., Sanzgiri, U., Dallas, C.E., and Bruckner, J.V. "Improvement of extraction efficiency in analysis of volatile organic compounds (VOCs) in tissues." 31st Annual Meeting of the Society of Toxicology, Seattle, WA; *Toxicologist* 12: 424, 1992.

Methods currently in use in quantitate VOCs in tissues have the inherent problem of volatilization of the compounds during processing. A new technique employing KOH dissolution was developed to increase the efficiency of VOC recovery. This approach was compared to a previous method involving tissue homogenization in saline and isoctane. Percent recoveries of trichloroethylene (TCE), 1,1,1-trichloroethane (TRI), carbon tetrachloride (CCl₄) and perchloroethylene from spiked tissues were determined for the two methods. A

specific amount of each VOC was injected into fresh, chilled samples of liver, kidney, fat, skeletal muscle, fat, heart, GI tract, spleen and brain taken from male S-D rats. Other rats were gavaged with an aqueous emulsion of 6 mg TRI/kg bw. The rats were sacrificed 10 min post dosing and the same organs removed. Tissue samples were treated in two ways: (1) diced in vials containing 2 ml 10N KOH and 8 ml isoctane, capped and allowed to stand for 1 hr, and vortexed; (2) homogenized in vials containing 2 ml saline and 8 ml isoctane. All of the samples were then centrifuged at 4°C at 1,800 x g for 10 min, and aliquots of the isoctane analyzed for VOC content by GC headspace analysis. Use of the KOH dissolution technique resulted in a higher % recovery from spiked samples than did the homogenization procedure for all 4 VOCs. Recovery of TCE by the KOH dissolution method, for example, ranged from 91% for fat to 105% for the GI tract. Recovery of CCl₄ using KOH was in the order of 95-100% for all tissues. TRI concentrations in tissues of rats gavaged with the VOC were consistently higher when the KOH procedure was employed. These findings indicate that KOH dissolution precludes the necessity of homogenizing tissues, and results in more efficient extraction of VOCs.

Male Sprague-Dawley rats (Charles River Labs, NC) in the weight range of 300-400 g were used for the study. Animals were housed in cages singly or in groups of two with a 12-hour light/dark cycle and *ad libitum* food and water. After ether anesthesia, the animals were exsanguinated by decapitation or closed cardiac puncture. Approximately 1 g of the following tissues were then excised (liver, kidney, perirenal fat, heart, muscle from thorax, brain and spleen). Tissue samples were placed into 20-ml scintillation vials containing 2 ml 10 N potassium hydroxide (KOH) and 8 ml gas chromatography (GC) grade isoctane. Compounds of interest were Trichloroethane (TRI); Trichloroethylene (TCE); Perchloroethylene (PER); Tetrachloroethane (TET); Carbon tetrachloride (CCl₄) were dissolved in isoctane to yield solutions of varying concentrations ranging from 0.1 mg/ml to 20 mg/ml. The tissues were spiked by injection of known quantities of the VOCs in isoctane (max volume 8 μ l), using a microliter syringe.

Parallel experiments were done to study the extraction efficiency a homogenization method vs the KOH method. Groups of 4-6 animals were dosed with the VOCs as a 5% aqueous Emulphor® emulsion, either intragastrically by gavage or intraarterially via a surgically implanted carotid cannula. The above said tissues were removed from the animals 5-10 in post dosing and processed as described above. The tissues were allowed to solubilize at room temperature in the tightly sealed headspace vials for periods of 30 min to 1 hr. Appropriate controls were run containing only KOH and isoctane or isoctane alone. The solubilization process was facilitated by mincing the tissues with a fine pair of scissors in the medium. Samples were then vortexed for 30 sec or longer to ensure that solubilization was complete and centrifuged at approximately 1800 x g for 10 min. A 10-ul aliquot of the supernatant was transferred into a headspace vial and capped with an aluminum cap and PTFE-lined septum. The vials were placed into a thermostatically heated chamber at 80°C and a portion of the vapor phase injected automatically into the column. The GC conditions were: 6' x 1/8" stainless steel column packed with 3 % OV 17 maintained at 80°C, injector port 150°C, electron capture detector 360°C. Standard curves were developed using isoctane solutions of VOCs over a linear range of 1 ng to 50 ng. The GC output area was used to compute the recovery of VOCs, based on the standard curve and the amount of the compound injected into each tissue. Appropriate controls were used to compensate for any VOC loss during processing.

Recoveries of TRI from the KOH-processed samples were fairly consistent across the range of concentrations used to spike tissues. The lowest recovery was seen from muscle at the lowest concentration utilized (75% @ 0.1 mg/ml). Generally there was an increase in the percent recovery as the TRI concentration increased in the spiked tissues (80-100%) (Table 1). A similar concentration-dependent pattern of recovery was seen in TCE-spiked tissues (Table 2). Percent recovery values were generally quite high (80-100%) at TCE concentrations \geq 0.2 mg/ml (i.e. ~1.6 μ g/g tissue). All the tissues exhibited consistent recovery values. Recovery from tissues of the *in vivo* dosed group did not differ significantly with the two techniques (Fig. 2). The amount of TCE in tissues of rats dosed orally with 5 mg/kg was relatively low. This can likely be attributed to the extensive metabolism of TCE vs. TRI and PER.

When the chemical was given intraarterially, it was distributed to all tissues indicating that a portion of the dose was not removed by presystemic elimination (i.e. the 6 mg/kg bolus dose exceeded the uptake/metabolic capacity of the liver, as well as presystemic elimination. Fat had a much greater amount of TCE than did other tissues. Substantial levels of TRI and PER were also found in fat, but liver levels were also quite high because of limited metabolism of the compounds. No statistically significant difference in TCE extraction was seen between the KOH and the homogenization method groups. Studies of tissues spiked with PER (1 mg/ml) showed good recovery, ranging from 85 to 92% (Table 3). Rats dosed orally with 10 mg PER/kg bw, showed relatively high levels of the chemical in liver, kidney and fat. Lower concentrations were measured in the other tissues. Amounts of PER extracted by the two methods were not significantly different, except in blood (Fig. 3). Carbon tetrachloride showed a very poor recovery when the chemical was given orally. Percent recovery with tissues spiked with CCl₄ yielded values comparable values to TCE, TRI and PER (i.e., high recovery). Studies with TET were less successful (data not shown). Recovery of TET from spiked tissues as well as *in vivo* studies was quite low. This can be attributed to a strong chemical interaction between TET with KOH, which effectively diminished the quantity of TET in biological systems.

The KOH extraction procedure yielded excellent recovery of TRI, TCE, PER and CCl₄ from spiked tissues (i.e., 80-100%). Percent recovery values were somewhat reduced for the lowest concentration of TCE and TRI. Recovery values were dose-independent when higher concentrations (i.e. 0.2-20 mg/ml) were utilized *in vitro*. The two methods yielded similar percent recovery values for tissues of animals dosed *in vivo*. Experiments will be conducted to determine whether this is true for spiked tissues. The new method described herewith has distinct advantages over the currently (i.e., homogenization) method in that it is time consuming and strenuous to process a large number of samples.

VI. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL DEVELOPMENT FOR INHALED PCE

An important goal of the project has been to develop and validate physiologically-based pharmacokinetic (PBPK) models, which will allow accurate prediction of the concentration of halocarbons in blood and tissues over time following inhalation and oral exposure. The pharmacokinetic studies conducted in earlier phases of the project have thus provided a unique data base from which to formulate and test the models. Data from the direct

measurements of blood and exhaled breath levels of halocarbon have been compared to simulated values calculated from mass-balance differential equations comprising the model. Thereby, the accuracy of the model has been tested by comparison to observed blood and exhaled breath concentrations.

The current investigation of the uptake and elimination of PCE in rats provided the first available data base for direct measurement of PCE in the exhaled breath and blood during inhalation exposures in rats (See Appendix A, Figures 2 and 3). A PBPK model was therefore developed to describe the disposition of PCE in the rat (Appendix A, Figure 1) using this unique opportunity for comparison of computer simulated values with these direct measurements for validation of the model.

A paper has been published in a scientific journal for these results. The reprint is included in this report as Appendix C, and the reference is as follows:

Dallas, C.E., Chen, X.M., O'Barr, K., Muralidhara, S., Varkonyi, P., and Bruckner, J.V. "Development of a physiologically based pharmacokinetic model for perchloroethylene using tissue concentration-time data," *Toxicology and Applied Pharmacology* 128: 50-59 (1994).

The pharmacokinetics of perchloroethylene (PCE) was studied in male Sprague-Dawley rats to characterize and quantify systemic uptake and respiratory elimination by direct measurements of the inhaled compound. Fifty or 500 ppm per was inhaled for 2 hr through a one-way breathing valve by unanesthetized rats of 325-275 g. Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected during and following PCE inhalation and analyzed by gas chromatography. PCE exhaled breath and alveolar levels increased rapidly after the initiation of exposure to near steady-state within about 60 min. They were then directly proportional to the exposure concentration. Uptake of PCE in the blood was also rapid, but blood levels continued to increase progressively over the course of the 2-hr exposure at both dose levels. Cumulative uptake, or total absorbed dose, was proportional to the inhalation exposure level.

A blood flow limited physiologically-based model was characterized with PCE eliminated in the exhaled breath and to a limited extent by liver metabolism. PCE concentrations in the blood and exhaled breath were well predicted by the PBPK model. The usefulness of model simulations in predicting systemically absorbed doses of PCE was demonstrated, which can have utility in risk assessments involving the internal dose of volatile organics.

VII. ESTABLISHING TET PARTITION COEFFICIENT PARAMETERS FOR PBPK MODEL IN DOGS

Physiologically-based pharmacokinetic (PBPK) models are a logical approach to improving the scientific basis for interspecies extrapolation in risk assessments of halocarbons and other volatile organic chemicals. In order to improve the accuracy of the model, predictions, tissue:blood partition coefficients were estimated using direct measurements of chemical TET in dog tissues and blood.

Animals were trained to stay calm and steady during experimentation, by getting them accustomed to sling, over a week's period. Dogs (6-9 kg) were utilized in these studies. For the animals intended to receive IA administration of the test compound, an indwelling jugular vein cannula was surgically implanted the day prior to the exposure. For procuring blood samples following TET administration, an indwelling carotid arterial cannula was implanted in the dogs. Both cannulas exited the body of the test animals behind the head, and the animals were allowed to recover from anesthesia until the following day. Food was withheld during the 18 hr recovery period before dosing.

The eight dogs were administered a loading dose of 5 mg/kg by jugular vein cannula over 1 min period, simultaneously infusion dose, at a rate of 28 $\mu\text{g}/\text{min kg}$, was started through the jugular vein. Both loading and infusion doses used polyethylene glycol (PEG) as a dosage vehicle. Infusion was for 4 hours. The IV administration was done using 1 ml gas tight Hamilton syringe. Serial 20 μl blood samples were taken at selected intervals from 2 min to 4 hours following dosing. The concentrations of TET in the blood samples were determined by headspace analysis using a Perkin-Elmer Sigma 300 gas chromatograph. At the end of 4 hours, the dogs were immediately euthanized by using pentobarbital and saturated potassium chloride, and sacrificed. Approximately 1 g tissues of liver, kidney, fat, heart, lung, muscle, and brain were collected and transferred into 20 ml chilled scintillation vials containing 2 ml of saline and 8 ml of isoctane. Tissues were homogenized, vortexed and centrifuged as before. Twenty ml of the organic phase was withdrawn with a pipet and transferred to 8 ml headspace vials to be analyzed in the Perkin-Elmer sigma 300 gas chromatograph. The highest peak of TET concentration appeared immediately following loading dose.

The steady state was attained at about 45 min after dosing (Fig. C-1). The concentration at steady state was 0.750 $\mu\text{g}/\text{ml} \pm 0.07$ which was close to projected concentration 1 $\mu\text{g}/\text{ml}$. The concentrations of blood and other tissues, at the end of 4 hours, are shown in table C-1. The result shown that highest concentration of TET was seen in fat tissue. In decreasing order were kidney, heart, brain, liver, lung and muscles. These parameters will be used for establishing tissue-blood partition coefficients of TET in dog which provide valuable input for halocarbon PBPK model parameter estimates.

VIII. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL DEVELOPMENT WITH PHYSIOLOGICAL PARAMETERS FOR DOGS IN PBPK MODELS

When an investigator is attempting to gather physiological parameters as input functions in pharmacokinetic models, he often finds a wide gulf in literature, regarding this vital aspect. An attempt was made to rectify this deficiency, so that future modelers will have a ready source of information in reviewing the available data. Attention has been given to the sources where, experimental conditions were totally artificial, contrary to normal physiologic function. The breed, the weight range and the sex of the animals was identified for these studies. The literature was searched and primarily those data that are relevant to our purpose have been included. This selection was based primarily on the weight of the dog and sex of the dog. As much as possible, effort has been made to list parameters with a fair amount of uniformity in reporting.

Some methods described here are classic approaches. Most of the studies for measuring tissue blood flow employed microsphere techniques with various radioactive nuclides. Other methods included Wetterer, electromagnetic flowmeter, Fick method and dye dilution. Some researchers have utilized ^{39}Fe to measure erythrocyte mass, ^{131}I to measure plasma volume for determining liver blood flow. Doppler flow probe is one of the latest devices used in measuring blood flow measurement. Carbonized plastic radioactive microspheres have been used for perfusion of muscle. Measurements of blood flow to fat is one of the most difficult procedures. People have attempted to use various methods, such as silicone oil drop chamber and ^{133}Xe -wash-out. Some of the blood flow measurements have used constant perfusion rates. This condition seems very artificial and may not be useful in predicting accurate blood flow to the adipose tissue. However, some researchers have used a somewhat physiological perfusion criterion, wherein the blood flow was maintained at 125 mm Hg which is closer to mean arterial pressure. So while compiling this kind of data from literature, it is imperative that thought be directed to the methods used in research and their applicability for a modeler in predicting/assessing the physiologic parameter. Newer and more sophisticated methods have been used for measuring blood flow which probably are more accurate. Irrespective of the method, used, the investigator is obligated to use the value, which most approximates his experimental setup, in order to get a better fit of the model, since, any small change in the input parameter may have a significant impact on the model prediction.

Method of Measurement:

Direct Fick: The concentration of oxygen in the venous and arterial bloods are measured chemically, and rate of oxygen adsorption by the lungs is measured by a respirometer through which the person breathes. Cardiac output can be calculated by comparing oxygen absorbed per minute by the lung with arteriovenous oxygen difference.

Dye-Dilution: A small amount of dye (i.e. Cardio-Green dye) is injected into a large vein, this then passes rapidly through the right heart, the lungs, the left heart, and finally into the arterial system. The concentration and duration of dye are recorded as it passes through one of the peripheral arteries for calculating cardiac output.

Electromagnetic flowmeter: Since blood is a conductor of electricity, the flow of blood with the vessel placed in a magnetic field generates an electric potential across the vessel in accordance with the principle of electromagnetic induction. The electromagnetic flowmeter has a frequency response more than adequate for the pulsatile wave forms found in the circulation. Its response is linearly proportional to the velocity of blood flow; calibration either in vivo or in vitro is necessary to convert its electric output into absolute units of velocity or volume flow per unit time.

Doppler Flow Probe: Transcutaneous Doppler flow probe reflect transit time of ultrasound wave from the moving blood to measure blood flow indirectly. The vessel is enclosed within the two halves of a cylinder with a crystal at each end, on opposite sides. These crystals act alternately as senders and receivers of a sound burst that passes diagonally across the vessel. The transit time downstream is shorter than upstream; from the difference between these electronically determined times, the volume flow through the vessel can be calculated.

Radioactive Microspheres: Regional blood flow were determined with microsphere 1.0- 1.5×10^6 NEN-TRAC microspheres with a normal diameter of $15\mu\text{m}$ were used, labeled with one of the following radionuclides: ^{46}Sc , ^{113}Sn and ^{153}Cd , which were injected into left atrial catheter. After the animals were killed, organs and tissues were cut into small pieces and counted, allowing determination of the blood flow corresponding to each isotope. Counting was performed in a three-channel Auto-gamma Packard with appropriate settings for each isotope.

Erythrocyte Mass ^{59}Fe & Plasma volume ^{131}I : The dog received an infusion of freshly drawn compatible dog red cell tagged with ^{55}Fe for determination of circulating red cell volume. At the same time, an infusion of bovine albumin of fresh dog plasma iodinated with radioactive iodine (^{131}I) was given intravenously. The unit quantity of total red cells was based upon the relative concentration of hemoglobin in whole blood and that obtained by extraction of hemoglobin from tissue samples. The unit quantity of cell sample drawn just prior to death, and the tissue. Rapidly circulating red cell content was based on ^{59}Fe measurements. Total plasma and red cell content was taken as the product of unit content and organ weight. Iodine measurements reflect only circulating plasma.

Silicone Oil Drop Chamber: Drop recording of blood flow is that the blood drops collect and fall through a colorless incompressible silicone fluid. Silicone is fully indifferent relative to blood, it can be contacted with blood directly. the blood flow was measured by cannulating the artery leading to the adipose tissue and directing the blood by plastic tube to a photoelectric drop (silicone oil) recorder, which in turn operates an ordinate recorder.

^{131}Xe - Wash-Out Method: Xenon, due to its high lipid solubility, can be assumed to cross the lipidcontaining cellular membrane freely. $^{133}\text{Xenon}$ dissolved in sterile isotonic saline solution was injected into subjects. The disappearance rate of the isotope was measured with a sodium-iodide crystal, local clearance of ^{133}Xe has been applied to blood flow measurement.

Carbonized Plastic radioactive microspheres: Carbonized plastic microsphere, $9 \pm 1\mu\text{m}$ in diameter, were labeled with gamma-emitting nuclides ^{141}Ce (specific activity 12.4 m Ci/g) or ^{85}Sr (specific activity 12.86 m Ci/g). The microspheres were suspended in 10% low molecular weight dextran solution to which one drop of Tween-80 had been added to minimize aggregation. After 30 min of mixing, three to four million microspheres (0.5-1.0 ml) were injected into the left atrium. All tissues were cut in 1-3 g samples, the activity of each nuclide was determined with a three-channel gamma-well counter. Raw counts were then corrected and compared with the arterial, reference blood sample to obtain blood flow expressed in milliliters per minute per gram.

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IX. DETERMINATION OF HALOCARBON PARTITION COEFFICIENTS FROM TISSUE CONCENTRATION-TIME DATA

Tissue and blood concentration-time data from intraarterial administration of PCE in male Sprague-Dawley rats was utilized to obtain tissue-to-blood partition coefficients and to compare to model predicted PCE concentrations. Blood flows and tissue volumes were obtained from literature values. A blood-flow limited model was utilized with PCE eliminated unchanged in the exhaled breath and by saturable liver metabolism. The model consisted of tissue compartments for liver, kidney, fat, heart, lung, muscle, brain, blood and the rest of the body. Overall, there was good agreement between predictions of PCE tissue concentrations over time by the PBPK model and the direct measurements of PCE in rat tissues. Relative to

other tissues, PCE muscle concentrations were initially overpredicted. Tissue area-under-the-concentration-time curves (AUCs) were predicted well with the PBPK model. In addition to their utility in developing PBPK model parameter estimates, tissue concentration time data were demonstrated to be useful in the validation of specific tissue compartments of a PBPK model for PCE by ia administration.

These results were presented at the most recent national meeting of the Society of Toxicology in Seattle, Washington. The reference for the abstract is as follows:

Dallas, C.E., Varkonyi, P., Chen, X.M., Muralidhara, S., and Gallo, J.M. "Validation of a physiologically based pharmacokinetic model of perchloroethylene in rats using tissue concentration-time data." 31st Annual Meeting of the Society of Toxicology, Seattle, WA; *Toxicologist* 12: 346, 1992.

Male Sprague-Dawley rats (325-375 g), obtained from Charles River Laboratories, were employed in these studies. For the animals intended to receive the ia dose, an indwelling carotid arterial cannula was surgically implanted the day before the exposure. The cannula exited the body of the test animal behind the head, and the animals were allowed to recover from anesthesia until the following day. For ia administration, PCE was administered at a dose of 10 mg/kg as an emulsion in polyethylene glycol (PEG) in a single bolus dose through the carotid arterial cannula.

Groups of animals ($n=4$) were serially sacrificed (using etherization) following ia dosing at the following time intervals = 5, 10, 15, 30, and 60 minutes, and 1, 2, 4, 6, 12, 24, and 36 hours. Blood samples were obtained by cardiac puncture. Approximately 1 gram each of liver, kidney, brain, lungs, heart, fat, and muscle were quickly removed and placed in 4 ml cold saline. Tissues were homogenized for the shortest possible time intervals, specific for each organ, to reduce the volatilization of the test compound during homogenization. The PCE in each sample was then extracted with 8 ml isoctane. A 20 μ l aliquot was placed in an 8 ml headspace vial, which was capped and subjected to controlled temperature and pressure conditions in a Perkin-Elmer HS-6 Headspace Sampler.

Analysis was made of the PCE in the vial headspace on an OV-17 (6' x 1/8") stainless steel column in a Perkin Elmer gas chromatograph with an electron capture detector. The column temperatures were: detector-360°C, column-150°C, headspace-140°C, injector-200°C. Values were compared to a standard curve, and the tissue concentration corrected for the percent recovery characteristic for each tissue.

Due to the highly lipophilic nature of PCE, the maximum tissue concentration (C_{max}) in the fat was substantially higher (ranging from 1.6 to 11.4 times greater) than the other tissues. The tissue with the lowest rate of blood perfusion of those sampled, muscle, had the lowest C_{max} . The relative importance of perfusion in the tissue pharmacokinetics of PCE provided additional verification to the perfusion-limited approach to a PBPK model for PCE.

The area under the tissue concentration-time curve (AUC) for fat tissues was between 34 and 67 times greater than the AUC for liver, kidney, heart, lung, muscle, and brain. The tissue AUCs for liver, kidney, and brain were very similar, reflecting their similarity in a high rate of blood perfusion. The predictions of tissue AUCs for PCE were generally within 5% of

the AUCs calculated from the observed tissue concentrations over time, except for liver and fat which had predicted tissue AUCs within approximately 9% of the observed tissue AUC.

In comparisons with observed tissue concentration-time data, tissue concentrations of PCE were well predicted by the PBPK model in brain, blood, kidney, heart, and lung over the length of the time course following PCE administration. In the first minutes following PCE exposure fat tissue levels were underpredicted, followed by fairly accurate predictions up to 84 hours, and terminal time points that were overpredicted relative to the observed fat PCE concentration. There was an initial over prediction of PCE concentrations in the muscle tissues up to 3 hours following administration, with more accurate predictions thereafter.

The PBPK model for PCE was therefore demonstrated to have considerable utility in accurately predicting tissue levels in rats. The least accurate predictions occurred in the most poorly perfused tissue sampled, muscle, and in the fat tissues more than 3 days following exposure. Tissue AUC proved to be a parameter accurately predicted by the PBPK model, which may be of significant utility in defining the applicability of this and similar models in risk assessments pertaining to specific compartments of the model.

X. TISSUE TIME COURSE KINETIC STUDIES OF PCE IN RATS FOR CORRELATION WITH NEUROBEHAVIORAL MEASUREMENTS

Exposures to volatile organic compound (VOCs) result from their widespread commercial use and improper disposal. VOC exposures may produce central nervous system (CNS) depressant effects. The intensity and time course of these CNS effects are assumed to be dependent upon the level of VOC in the brain. The relationship between brain dose and neurobehavioral response must be inferred from animal studies. PBPK models were developed to describe a chemical's dynamics in the blood and specific organs. Furthermore, chemical's effects are being quantified and used to establish biological response or toxicodynamic models. Once the relationship between brain concentration and neurobehavioral toxicity is established a toxicodynamic model can be developed to predict human neurobehavioral effects for a given chemical exposure.

Male Sprague-Dawley rats (300-350 g) were maintained on a restricted diet (10 g/day) for 72 hours prior to being surgically implanted with an indwelling carotid artery cannula. After an overnight recovery period, rats received 1 ml of oral bolus of either 160 or 480 mg/kg PCE in a 10% aqueous Emulphor® emulsion. Serial blood samples were collected via the carotid artery cannula at 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, and 90 min up to 72 hours.

Male Sprague-Dawley rats (300-350 g) were maintained on a restricted diet (10 g/day) for 72 hours prior to receiving a 1 ml oral bolus of either 160 mg/kg PCE in a 10% aqueous Emulphor® emulsion or 480 mg/kg PCE in a 20% aqueous Emulphor® emulsion. Animals were serially sacrificed by decapitation at 1, 6, 12, 15, 20, 30, 40, 50, 60, and 90 minutes post dosing. Samples of brain, liver, fat and muscle were removed immediately and placed in chilled scintillation vials containing 2 ml of saline and 8 ml of isoctane. Tissues were homogenized, vortexed, and centrifuged. Blood samples and aliquots of the tissue

supernatant were diluted in saline and placed in 20 ml headspace vial analyzing in Perkin-Elmer Model 8500 gas chromatograph.

Absorption of PCE following oral administration was relatively slow in rats, with peak blood concentration of 45 min and 105 min following 160 and 480 mg/kg dosing respectively. The maximum concentration of PCE in the blood for 160 mg/kg dose was 24.17 μ g/ml; and at 480 mg/kg dose was 89 μ g/ml. The half-life of PCE in both doses were relatively close, 8.4 hrs and 9.25 hrs respectively. Following oral administration of PCE, AUC of 480 mg/kg dose was 2.65 times as high as AUC of 160 mg/kg dose.

In the tissue studies of both 160 mg/kg and 480 mg/kg up to 90 min, all tissues show steady state levels from 15 min onward until termination. The AUC for fat was highest, and in the following order were liver, brain, muscle, and blood. The result shows that brain concentrations were lower than in fat and liver.

XI. NEUROBEHAVIORAL MEASUREMENTS IN RATS DURING AND FOLLOWING PCE AND TRI EXPOSURE

One objective of the project is to develop a combined physiological pharmacokinetic-toxicodynamic model that would allow the prediction of behavioral toxicity given brain concentrations derived from a validated physiologically-based pharmacokinetic model. To this end, we are currently attempting to define the relationship between the brain concentration of a particular halocarbon and the resulting behavioral toxicity. Thus far, our efforts have been concentrated on halocarbon-induced behavioral impairment in rats as measured in various operant paradigms. Halocarbons have been administered by oral gavage as well as by inhalation.

A dose-range finding study was conducted in which male Sprague-Dawley rats were administered Perchloroethylene (PCE) as a single oral bolus, after which their behavior was evaluated under a fixed-ratio 40 schedule of reinforcement for 90 minutes. To a lesser extent, the study evaluated the effect of various dosing vehicles and training time on PCE-influenced behavior. Additionally, the concentration of PCE in blood, brain, liver, fat and muscle was determined for up to 90 minutes following oral dosing with either 160 or 480 mg/kg PCE. The study's results were presented in part in poster form at the latest meeting of the Society of Toxicology that was held in Seattle, Washington in February 1992. The reference for this abstract is as follows:

Warren, D.A., Dallas, C.E., Reigle, T.G., and Muralidhara, S.: "Neurobehavioral Toxicity of Ingested Perchloroethylene in Rats." 31st Annual Meeting of the Society of Toxicology, Seattle, Washington: *The Toxicologist*: Vol.12, No.1 (1992).

Based upon the results of the above study, a larger study was conducted in which male Sprague-Dawley rats were administered a single oral bolus of either 160 or 480 mg/kg PCE, after which their behavior was evaluated under a fixed-ratio 40 schedule of reinforcement for 90 minutes. This study resulted in a behavioral data set that can be interpreted with full knowledge of blood and brain levels of PCE at various times during the operant session. Although the interpretive phase of the study is not yet complete, it is clear that the operant

paradigm employed was sensitive to the animals' dose-dependent behavior. It is apparent that the administration of a vehicle placebo did not result in deviations from normal behavior beyond that expected from inter-day variability in the animals' response rates. Likewise, a dose of 160 mg/kg PCE did not result in behavioral depression, whereas 480 mg/kg as a behaviorally depressive dose of PCE from which recovery was gradual as the operant session progressed.

In another study, an operant test cage was placed inside an inhalation chamber so that behavior could be monitored concurrent with exposure. The study was undertaken to examine the feasibility of cumulative dosing by inhalation. The use of cumulative dosing would be of great benefit in establishing dose-response relationships due to the time that must be expended to shape and train experimental animals. Male Sprague-Dawley rats were allowed to establish a baseline response rate for 40 minutes under a variable interval-60 schedule of reinforcement. After 40 minutes, the animals were exposed to either 1000, 2000, 3000, 4000, or 5000 parts per million Trichloroethane (TRI) for 100 minutes. Forty-eight hours after exposure, the absence of any residual behavioral effect was verified. Seventy-two hours after exposure, each animal was exposed to a different concentration of TRI, until each animal had been exposed to all five concentrations. A cursory look at the data reveals that cumulative dosing with TRI by inhalation may not be feasible, as one TRI exposure may influence behavior during subsequent exposures. This appears to be the case even though bioaccumulation of TRI does not occur under the exposure schedule employed. Sudden deviations from linearity upon initiation of exposure indicates that the animals may be sensitized to the odor and/or irritant properties of TRI (Figure E-18). Defining an accurate dose-response relationship using cumulative dosing by inhalation may also be hindered by inter-day variability in response rates.

As an alternative to cumulative dosing, a study is underway in which animals are exposed to only a single concentration of TRI. Male Sprague-Dawley rats are exposed to a single concentration of TRI from 1000 to 5000 parts per million by inhalation, during which time behavior is measured under a variable-interval 30 schedule of reinforcement. As expected, rats maintain a stable rate of baseline responding over the duration of the operant session resulting in a linear, and thus predictable, cumulative response curve. We believe that a quantitative measure of halocarbon-induced behavioral impairment may be obtained by measuring an animals' deviation from linearity under exposure conditions. The magnitude of deviation from linearity occurs in a dose-dependent manner. Upon completion of the behavioral study, the uptake of TRI into tissues and blood will be measured during exposures to concentrations equivalent to those employed in the behavioral study. If the magnitude of deviation from linearity is found to correlate with the brain concentration of TRI, this would represent major progress toward the development of a toxicodynamic model predictive of halocarbon-induced behavioral changes. It is expected that preliminary data from this study will be presented at the next Annual Meeting of the Society of Toxicology in New Orleans, La.

XII. EVALUATION OF PBPK MODEL PREDICTIONS OF PCE UPTAKE AND RESPIRATORY ELIMINATION

The objective of this phase of the project is to evaluate the accuracy of the PBPK models, which allows prediction of the concentration of halocarbons in exhaled breath and tissues over time following inhalation exposure. The pharmacokinetic studies conducted in the project provide a unique data base from which to test the model. Data from the direct measurements of exhaled breath and blood concentrations have been compared to simulated values calculated from mass-balance differential equations comprising the model. Thereby, the accuracy of the model is being tested and adjustments made where necessary to improve the model simulations.

Toward this end, the pharmacokinetics of perchloroethylene (PCE) was studied in male Sprague-Dawley rats to characterize systemic uptake and respiratory elimination by direct measurements of the inhaled compound. Fifty or 500 ppm PCE was inhaled for 2 hr through a one-way breathing valve by unanesthetized male Sprague-Dawley rats of 325-375 g. Serial samples of separate inhaled and exhaled breath streams, as well as arterial blood, were collected during and following PCE inhalation and analyzed by headspace gas chromatography (GC).

The studies involved in the testing of the PBPK model have been included in a paper published in *Toxicology and Applied Pharmacology*. The manuscript is included as Section D of the Appendix, and the citation is as follows:

Dallas, C.E., Muralidhara, S., Chen, X.M., Ramanathan, R., Varkonyi, P., Gallo, J.M., and Bruckner, J.V. "Use of physiologically-based model to predict the systemic uptake and respiratory elimination of perchloroethylene." *Toxicology and Applied Pharmacology* 128: 60-68 (1994).

As part of the respiratory elimination studies to be conducted in the current proposal, respiratory volumes were directly measured. In addition to the calculation of cumulative uptake during exposure and elimination following exposure, monitored respiratory volumes were useful in providing accurate values for use in the PBPK models. Substantial variations in the magnitude of respiratory parameters can occur in the same species due to stress, age differences animal care procedures, variability in animals provided by different vendors, and differences in respiratory monitoring techniques in different laboratories. Thus, the use of published values for input into the PBPK models may lead to inaccurate predictions. The magnitude of the administered dose and quantity of compound exhaled during inhalation exposures are highly dependent on the rate and volumes of respiration since exhalation is the primary route of elimination for PER and TET.

Parameters for a blood flow limited physiologically-based model were determined from tissue concentration-time data in the manuscript in Appendix B, and a model characterized with PCE eliminated in the exhaled breath and to a limited extent by liver metabolism. PCE concentrations in the blood and exhaled breath were well predicted by the PBPK model.

The ability of the current PBPK model to predict disposition of PCE increases the confidence in the utility of the model in health risk assessments. The use of a tissue

concentration-time data for establishing PBPK model parameters was found to be helpful in developing a model to provide accurate predictions both during and following inhalation exposure.

XIII. INTERSPECIES COMPARISONS IN PBPK MODEL PARAMETERS ESTIMATION

Similarities in the anatomy and physiology of mammalian species make interspecies extrapolations (i.e. animal scale-up) possible. Models developed in a series of species may be scaled based on allometric relations to allow prediction of chemical concentrations in other species without the collection of additional experimental data. Model parameters such as tissue volumes and blood flows can be scaled based on allometric relationships which are functions of animal weight. The ability to scale-up animal physiological models to humans is a powerful tool to obtain predictions of tissue chemical concentrations in humans.

One need in development of the PBPK model for use in different species are tissue-blood partition coefficients. Absorption rates, metabolism constants as well as other model input parameters, of course, these parameters are likely to vary considerably between species.

The tissue disposition of perchloroethylene (PCE) was determined experimentally in two mammalian species of markedly different size, in order to obtain model input parameters for in vivo data for the development of a physiologically based pharmacokinetic (PBPK) model to forecast the disposition of PCE in each species. Male Sprague-Dawley rats and male beagle dogs received a single bolus of 10 mg PCE/kg bw in polyethylene glycol 400 by gavage. Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, perirenal adipose tissue, and blood were taken for up to 48 hr following PCE administration. Blood and tissue PCE concentrations were analyzed using a gas chromatography headspace technique. While the blood:air partition coefficient was similar to that value determined using *n* *vitro* data in the literature, tissue:blood partition coefficients were 1.4 to 2.8 times greater for the in vivo data in this study compared to the available published *in vitro* values. The PCE blood:air partition coefficient for the dog was twice that of the rat, and the tissue:blood partition coefficients were 1.5 to 3.0 times greater in the rat relative to the dog.

In the development of these PBPK models, one of the most important model parameters is the tissue:blood partition coefficient. This value governs the rate of the transfer of the chemical between the blood and each of the tissue compartments represented by the model. An in vivo approach to deriving tissue:blood partition coefficients for PBPK models has been described, in which the tissue-concentration time course of the test chemical is employed. While this approach provides an in vivo description of the rate of transfer of the chemical for each tissue for which the data is available, there has been little opportunity for its use because of the paucity of detailed tissue concentration time data for most VOCs, including PCE. Therefore, the time course of uptake, disposition, and elimination of PCE in blood and seven tissues was determined in two species, and the data utilized in deriving partition coefficients for a PBPK model for PCE.

These investigations describing PBPK model estimation in rats and dogs for PCE have been published in a peer-reviewed journal. This paper is presented as section E of the Appendix, and the citation is as follows:

Dallas, C.E., Chen, X.M., Muralidhara, S., Varkonyi, P., Tackett, R.L., and Bruckner, J.V. "Use of tissue disposition data from rats and dogs to determine species differences in input parameters for physiological model for perchloroethylene." *Environmental Research* 67: 54-67 (1994).

Where available, in vivo tissue concentration time data can help to evaluate in vitro data, contribute to the design of tissue compartments of PBPK models, and test important assumptions in interspecies scaling of the models.

XIV. UTILITY OF PBPK MODEL PREDICTIONS BETWEEN SPECIES, DOSES, AND ADMINISTRATIVE ROUTE

In order to test how robust the PBPK model is at this point of development, it is useful to test the predictive ability under a variety of experimental conditions.

Pharmacokinetic models developed in a series of species may be scaled based on allometric relations to allow prediction of chemical concentrations in other species without the collection of additional experimental data. Model parameters such as tissue volumes and blood flows can be scaled based on allometric relationships which are functions of animal weight. Changing the input parameters for the concentration of chemical entering the exposed species allows for extrapolations between dose. Altering the point of entry of the chemical into the PBPK model enables pharmacokinetic comparisons between routes of administration using an otherwise similar modeling approach.

A primary objective of the current study was to evaluate the accuracy of a PBPK model in predicting blood levels of PCE between species, exposure concentration and route of administration in comparison to observed data from matched experiments conducted for that purpose. Where possible, equivalent doses were administered in two species of wide variation in size, the rat and the dog, and a variety of doses used in each species. These exposures were repeated using two routes of administration, and venous blood levels over time were used to document the time course of PCE uptake, disposition, and elimination under each of the experimental regimens. The utility of the PBPK model predictions were then evaluated under a variety of experimental conditions.

A paper using the PBPK model for simulations in both species has been published. The paper is included as section F of the Appendix, and the citation is as follows:

Dallas, C.E., Chen, X.M., Muralidhara, S., Varkonyi, P., Tackett, R.L., and Bruckner, J.V. "Physiologically based pharmacokinetic model useful in predictions of the influence of species, dose and exposure route on perchloroethylene pharmacokinetics." *Journal of Toxicology and Environmental Health* 44: 301-317 (1995).

It was demonstrated that the PBPK model is accurate in many aspects of the experimental conditions tested but some problems exist. Predicted concentrations in venous blood across a 10-fold range of doses for ia administration were accurate in rats. In dogs, however, there was some overprediction of PCE concentrations between the two doses tested.

XV. PBPK MODEL PARAMETER ESTIMATION FOR TET USING TISSUE CONCENTRATION TIME COURSE DATA IN RATS AND DOGS

The PBPK model development completed for PCE in the two species was then extended to TET.

As a class of chemicals, halocarbons have low solubility in blood and high volatility (i.e., low blood:air partition coefficients), as well as rapid vascular-alveolar transfer. Thus, a substantial proportion of the blood's burden of halocarbons should be removed during each pass through the lungs. It follows that halocarbons with low blood:air partition coefficients should be more efficiently eliminated and have a less prolonged CNS depressant action, than halocarbons with relatively high partition coefficients. In order to test this PREMISE, tetrachloroethylene (PCE) and 1,1,2,2-tetrachloroethane (TET) were utilized. Both PCE and TET are poorly metabolized (Ikeda and Ohtsuji, 1972) and have similar oil:blood (i.e., fat/blood) partition coefficients, but PCE has a much lower blood:air partition coefficient.

The ability of the PBPK model to accurately predict both PCE and TET pharmacokinetics would demonstrate the capacity of the model for halocarbons of a wide variation in volatility.

The studies for TET PBPK model parameter estimation were presented at the 1993 meeting of the Society of Toxicology. The citation for the abstract is as follows:

Dallas, C.E., Chen, X.M., Muralidhara, S., Varkonyi, P., Tackett, R.L., Gallo, J.M., and Bruckner, J.V. "Determination of physiologically-based model partition coefficients from 1,1,2,2-tetrachloroethane (TET) tissue pharmacokinetics." 32nd Annual Meeting of the Society of Toxicology, New Orleans, LA; Toxicologist 13: 173, 1993.

In order to improve the accuracy of physiologically-based pharmacokinetic (PBPK) model predictions, tissue:blood partition coefficients were estimated using direct measurements of tetrachloroethane (TET) in rat and dog tissue and blood. Male Sprague-Dawley rats received a single bolus of 10 mg/kg of TET in polyethylene glycol by oral gavage or by intraarterial administration through an indwelling carotid arterial cannula. Male beagle dogs received a single intraarterial bolus of 10 mg/kg of TET. In order to establish a steady-state equilibrium in test animals, other male beagle dogs were given a loading dose of 5 mg/kg via a jugular vein for 4 hours.

Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, adipose tissue, and blood were taken up to 24 hr following TET administration from the animals in each of these test groups. Percent recovery of TET from tissues was also determined for rats and dogs, using isolated tissues spiked with known concentrations of TET. Blood and tissue concentrations were analyzed for TET content using a GC-ECD headspace technique.

Percent recovery ranged from 87.8% from rat skeletal muscle to 95.7% from dog heart. There were no significant differences in % recovery between the two species. Considerably longer half-lives in the tissues and blood of dogs relative to an equivalent PCE administration in rats were found. Overall, values predicted by the PBPK model were in close agreement

with measured values in the nonfat tissues. following an equivalent dose the larger of the two test species had a larger area under the tissue concentration (AUTC) curve for all tissue compartment except kidney than the smaller species. These results will be helpful in the further investigation of some of the uncertainties in the application of tissue dose metrics in interspecies extrapolations of TET using PBPK models.

Male beagle dogs (8-14 kg), obtained from Marshall Farms, and male Sprague-Dawley rats (325-375 g), obtained from Charles River Laboratories, were employed in these studies. For the animals intended to receive the ia dose, an indwelling carotid arterial cannula was surgically implanted the day before the exposure. The cannula exited the body of the test animal behind the head, and the animals were allowed to recover form anesthesia until the following day. For ia and po administration, TET was administered at a dose of 10 mg/kg as an emulsion in polyethylene glycol (PEG) in a single bolus dose through the carotid arterial cannula or by oral gavage.

Groups of 4 rats and 3 dogs were serially sacrificed (using etherization) following dosing at the following time intervals = 5, 15, 30, and 45 minutes, and 1, 1.5, 2, 3, 4, 12, 16, 20, 24, and 36 hours for rats and 0.5, 2, 4, 8, and 12 hours for dogs. Blood samples were obtained by cardiac puncture. For establishing steady-sate equilibrium, dogs received a loading dose of 5 mg/kg through a jugular vein cannula. The animals then received a constant infusion of 28 μ g/min/kg through the cannula, and blood levels were monitored for TET over the next four hours. Once the blood levels remained constant for at least 2 hours, it was assumed that steady state had been achieved. Tissues isolated from unexposed animals were spiked with 0.1, 1, 5, 10, and 20 mg TET/g of tissue and the percent recovery for each tissue in each species established.

Approximately 1 gram each of liver, kidney, brain, lungs, heart, fat and muscles were quickly removed from all animals and placed in 4 ml cold saline. Tissues were homogenized for the shortest possible time intervals, specific for each organ, or reduce the volatilization of the test compound during homogenization. The TET in each sample was then extracted with 8 ml isoctane. A 20 μ l aliquot was placed in an 8 ml headspace vial, which was capped and subjected to controlled temperature and pressure conditions in a Perkin-Elmer HS-6 Headspace Sampler.

Analysis was made of the TET in the vial headspace on an OV-17 (6' x 1/8") stainless steel column in a Perkin Elmer gas chromatograph with an electron capture detector. The column temperatures were: detector-375°C, column-120°C, headspace-90°C, injector-200°C. Values were compared to a standard curve, and the tissue concentration corrected for the percent recovery characteristic for each tissue.

Due to the highly lipophilic nature of TET, the maximum tissue concentration (Cmax) in the fat was substantially higher (ranging from 7-28 and 11-48 times greater for rats and dogs, respectively) than the other tissues. The t_{1/2} and AUC was also much longer in the fat than in the other tissues for both species and both routes of administration. The AUC for each of the dog tissues was substantially greater than that of the rat tissues. The t_{1/2} and Cmax values were also greater for each tissue in the dog than in the rat. The elimination t_{1/2} for the brain was longer than in all other non fat tissues in the dog, but was shorter than the other non fat tissues in the rat following an equivalent ia administration. The poorly perfused

tissues (muscle) had the lowest AUC and Cmax for both po and ia administration in the rat, but was higher than other non fat tissues in the dog.

In comparisons with observed tissue concentration-time data, tissue concentrations of TET were well predicted by the PBPK model in brain, blood, kidney, heart, and lung over the length of the time course following TET administration. Following po administration in rats and dogs following ia administration were well predicted. There was some overprediction of muscle concentrations of TET in the dog by the model. In both species and routes of administration, there was a tendency for underproduction of the TET concentration during part of the time course. Following an equivalent ia exposure, the underproduction occurred in the later time points for rats and in the earlier time points for the dog.

Partition coefficients determined from the AUC for dog tissues following ia administration of TET were similar to values calculated from the steady-state determinations in tissues for brain, heart, and fat. There was more than a two-fold difference (2.0-2.8) between the partition coefficients for kidney, lung, and muscle calculated by the two approaches. Using the partition coefficients calculated using the AUC method for ia exposures, the PBPK model for TET was therefore demonstrated to have considerable utility in accurately predicting tissue levels in rats and dogs.

XVI. EVALUATION OF THE ACCURACY OF PBPK MODEL PREDICTIONS FOR TET ACROSS DOSES, SPECIES, AND ROUTE OF ADMINISTRATION

An important consideration in health risk assessments of halocarbon solvents is the validity of species to species comparisons in the uptake, disposition, and elimination of the chemicals following ingestion. Therefore, the relative toxicokinetics between species of wide variation in size was evaluated following tetrachloroethane (TET) ingestion. Male Sprague-Dawley rats and beagle dogs were administered TET at dose of 10 or 30 mg/kg. The halocarbon was administered in polyethylene glycol (PEG) in a single bolus either orally (po), or by intraarterial administration (ia) through an indwelling carotid arterial cannula. Blood samples were collected from an indwelling cannula in the jugular vein at intervals up to 48 hours following administration, and the halocarbon concentrations analyzed by headspace gas chromatography. The terminal elimination half-lives of TET in dogs were significantly longer than in rats for both routes of administration and for both po doses. Bioavailability, peak blood levels and the area-under-the-blood-concentration-time curves were also higher in dogs relative to equivalent dose in rats po and ia. These results show that there are significant species differences in the toxicokinetics and bioavailability of ingested TET, and that further evaluations are needed for making interspecies comparisons of toxicokinetic data for halocarbons.

Male beagle dogs (5-10 kg), obtained from Marshall Farms, and male Sprague-Dawley rats (325-375 g), obtained from Charles River Laboratories, were employed in these studies. For the animals intended to receive ia administrations of the test compounds, an indwelling carotid arterial cannula was surgically implanted the day prior to the exposure. For procuring blood samples following halocarbon administration, an indwelling jugular vein cannula was implanted in all the test animals. Both cannulas exited the body of the test animal behind the head, and

the animals were allowed to recover from anesthesia until the following day. Food was withheld during the 18 hr recovery period before dosing.

The rats and dogs were administered a single bolus dose of either 10 or 30 mg/kg TET, using polyethylene glycol (PEG) as a dosage vehicle. Both oral doses were administered using a gavage needle for rats and a teflon tube for dogs. The ia administration was conducted using the carotid arterial cannula. While rats exhibited no neurobehavioral effects following TET administration, the dogs receiving the 30 mg/kg ia dose demonstrated a very high degree of central nervous system (CNS) depression. Data for ia administration in dogs is therefore presented only for the 10 mg/kg dose.

Serial 20 μ l blood samples were taken at selected intervals for up to 48 hrs following dosing. The concentrations of TET in the blood samples were determined by headspace analysis using a Perkin-Elmer Sigma 300 gas chromatograph equipped with an electron capture detector and an automatic headspace analyzer. The operating conditions for the 6-ft x 1/8-inch stainless steel column were: headspace sampler temperature, 100°C; injection port temperature 200°C; column temperature, 140°C; detector temperature, 400°C; column packing, OV-17; flow rate for argon/methane carrier gas, 60 ml/min.

The blood concentration-time data were evaluated by R-strip (Micromath Scientific Software) and Lagran (M. Rocci and W.J. Jusko) computer programs for the assessment of the appropriate pharmacokinetic model and calculation of relevant pharmacokinetic parameters.

PBPK model predictions for TET in rats and dogs can be found in the Appendix, Section G. PBPK model prediction of TET in the venous blood of rats were well predicted at the 10 mg/kg dose, but underpredicted over the course of the 30 mg/kg dose following both ia and po administration. Predictions of TET predictions in dogs following oral administration were accurate over most of the time course for both doses. Predictions of TET concentrations in dogs following ia administration were not well predicted, especially in the terminal elimination phase.

Absorption of TET following oral administration was very rapid in rats and dogs, with peak blood levels achieved in both species and at both doses between 12 and 22 minutes. The maximum concentration of TET reached in the blood was higher in dogs than in rats in all cases, though this difference was only statistically significant for intraarterial administration. The half-life of TET was longer in the blood of dogs than in rats, and at a high level of significance by intraarterial administration and at both doses given orally. Following both oral and intraarterial administration of TET, AUC was significantly higher in dogs than in rats. The bioavailability of TET from oral exposure was higher in dogs than in rats. Following equivalent ia doses of 30 mg/kg TET, dogs exhibited severe CNS depression (including unconsciousness) while rats demonstrated no appreciable neurobehavioral effects. No CNS effects were observed in either species following po administration at either dose.

XVII. EVALUATION OF NEUROTOXICITY OF PERCHLOROETHYLENE USING ROTAROD AND COMPARISON WITH BLOOD AND BRAIN LEVELS

The rotarod has been in use since the mid 1950's, primarily as a tool to evaluate the effects of neurotoxins. Since its introduction there have been a wide variety of modification to the basic concept of the rotarod test. The rotarod has been used by several researchers to evaluate the neurotoxicity of solvents. The purpose of this study was to measure the effects of PCE on the rotarod performance in dynamically exposed animals during testing, and to attempt to correlate performance on the rotarod to blood and brain concentrations of perchloroethylene.

A dowel of 1 1/4 inch diameter and approximately 40 inches in length was adapted to rotate 13" above the floor of Rochester type dynamic flow chamber. The rod was divided into three 7 inch compartments by 4 cardboard disks 13 inch in diameter. The rod was driven by an externally mounted, variable speed motor supported outside of the chamber and attached directly to the rod via a side portal. For this experiment a speed of 10 rmp was used.

1,1,2,2 Perchloroethylene (PCE) (tetrachloroethylene) was obtained from Aldrich Chemical Company (Milwaukee, WI). Test atmospheres of PCE were generated by vaporization of the compound in different combinations of air flow and temperature control schedules. Approximately 1 liter of liquid chemical was placed into a glass dispersion flask. Nitrogen was passed through the dispersion flask and was directed into the chamber. A heating mantle was placed around the dispersion flask, and narrowly controlled temperature limits maintained for volatilization continuity. The entire halocarbon generation system was enclosed in a specially fabricated safety box. The box was maintained under constant negative pressure during inhalation exposures in the same manner as the inhalation chamber. Exhaust air from both the safety box and the inhalation chamber was vented to HEPA and charcoal filter, so that all chemical was scrubbed before release to the environment. Concentrations were monitored inside of the chamber by using a Foxboro Miran 1b ambient air analyzer.

Eight to 12 CD-1 male albino mice, weighing 35 to 40 grams were obtained from Charles River Laboratories (Raleigh, NC). All mice were maintained on constant light-dark cycle with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. Mice were housed in plastic cages, with tap water and Purina Mouse Chow #5010 provided ad libitum. Training sessions were begun after at least a 6 day acclimation period.

Animals would be placed on the rotarod and performance time monitored using an electronic stopwatch. If animals fell during the training session, their performance time would be noted and the animals were immediately placed back onto the rotarod. Training sessions for each animal would last a maximum of one hour per day. If the animal had successfully maintained itself on the rotarod for one hour, the animal was removed by the tail and returned to its housing. The animals were trained a total of six to eight days. After this training period, animals which produced a two day average performance of 30 minutes or less over the two days prior to exposure were removed from the study group. Only one animal was eliminated using this criteria. Most animals gave a two day base line performance of 40 to 60 minutes (Figure 1).

After 24 hours from the completion of training, a concentration of 1500 ppm, 2000 ppm, or 3000 ppm Perchloroethylene was generated and stabilized in the dynamic exposure chamber. The animal was placed on the rotarod via a portal door and performance was monitored using an electronic stopwatch. The animal was immediately removed from the chamber via the portal when it fell from the rotarod, and sacrificed using cervical dislocation. Approximately .2 ml blood was collected by cardiac puncture, and whole brain was removed from the animal. Both tissues were quickly placed into previously chilled 20 ml glass scintillation vials containing 4 ml of isoctane and 1 ml saline solution. Each tissue was homogenized with a Ultra-Turrax SDT homogenizer (Tekmar Co., Cincinnati, OH). Homogenization was accomplished as quickly as possible to minimize loss of the compound due to volatilization. The homogenates were then centrifuged at 1800 x gravity for 10 minutes at 4 degrees centigrade in capped scintillation vials. An aliquot of the isoctane layer was diluted with pure isoctane and transferred to 20 ml headspace vials. These vials were capped with latex rubber septa and crimped to ensure an airtight seal.

Each sample vial was then placed into the autosampler unit of a Perkin Elmer 8500 Gas Chromatograph (Norwalk CT). The following conditions were maintained on the G.C.: column oven temperature, 90°C; injector temperature 120°C; detector temperature 120°C. The analysis was carried out using a 6' x 1/8" stainless steel detector column packed with 3% ov-17 (80-100 mesh) Carrier gas was argon/methane @60 ml/min. Data analysis was done using Lotus 1-2-3 with a standard curve run on everyday of analysis.

Each animal's performance time on the rotarod was compared to the 2 day baseline average from the last two days on training. Animals responded to the perchloroethylene exposures in a dose dependent manner (Figure 1). Blood and brain Perchloroethylene concentrations were averaged and compared with exposure levels (Figure 2).

Although this test did successfully generate a dose response curve with PCE, it is difficult to interpret blood and brain concentrations of PCE at the time of fall and performance on the Rotarod. There are some significant limitations with this test method. Another factor is the significant limitations with this test method. One factor is the significant amount of training required to attain the baseline performance time on the rotarod. From this dose response curve, there is little justification for extending exposure below the 1500 ppm mark or above the 3000 ppm mark. The accelerod has apparently produced a more sensitive testing with alcohol and acrylic oral dosages in rats. However, the accelerod was not chosen because it would introduce an extra variable into the experimental schedule. We also are currently generating a pharmacokinetic model of Perchloroethylene in mice to confirm the blood and brain levels measured with the rotarod mice and to examine the possibility that performance on the rotarod could be related to other factors such as rate of uptake of compound into brain and subsequently the degree of neurological impairment.

Table 1

Animal Baseline Performance and number of days trained

XVIII. CORRELATION OF NEUROBEHAVIORAL EFFECTS WITH TISSUE AND BLOOD PHARMACOKINETICS OF TRI IN RATS

Neurobehavioral tests are useful in assessing the acute central nervous system (CNS) effects of short-term inhalation exposures to VOCs. It is important to assess changes in CNS basal activity in a valid quantitative manner, especially when considering interspecies extrapolations. Repetitive, on-line determinations of neurobehavioral response concurrent with solvent exposure are of significant utility in elucidation of the time-course of CNS effects of the compounds. Operant performance measurements have been found to be useful for the detection of subtle CNS effects of low VOC exposure levels , prior to reaching a level which would result in irreversible neuropathological changes. CNS-depressant effects of VOCs have been demonstrated in animals by operant tests at doses similar to doses that have been shown to alter human performance.

The purpose of this study was threefold: to determine the effect of inhaled TRI on operant responding; to measure the concentration of TRI in blood and tissues during inhalation exposure; and to examine relationships between blood and brain concentrations of TRI and changes in operant behavior. Male, Sprague-Dawley rats (275-325 g) were trained to lever press for an evaporated milk reinforcer (0.08 ml) on a variable interval 30 (VI 30)schedule for 2 hr. Trained rats were exposed to clean air for 20 min, then to a single concentration of TRI (500-5000 ppm) for 100 min. The number of operant responses in each 5 min interval for the operant session was recorded. Dose-response curves were generated by plotting TRI concentrations against both mean response ratios (TRI/control) and differences in the areas under the predicted and observed cumulative response curves.

These studies of the neurobehavioral effects of PCE following inhalation exposure were presented at the 1993 meeting of the Society of Toxicology. The citation for the abstract is as follows:

Warren, D.A., Dallas, C.E., Reigle, T.G., and Christmus, W. "Behavioral Changes During 1,1,1-trichloroethane (TRI) Inhalation in Rats: Relationship to brain and blood levels." 32nd Annual Meeting of the Society of Toxicology, New Orleans, LA; Toxicologist 13: 248, 1993.

Inhalation of 500 and 1000 ppm TRI did not decrease the rate of lever pressing, whereas 2000, 3500, and 5000 ppm TRI dose-dependently suppressed operant responding in the absence of any apparent motor impairment. Additional rats were exposed under the same conditions as those used in the behavioral study and were sacrificed at various times during exposure for blood and tissue collection. The relationships between blood and brain concentrations of TRI and changes in operant responding were examined by plotting blood and brain concentrations against mean response ratios and fitting the scatter plots with second order regression lines. Correlation coefficients for the relationships between blood and brain concentrations and operant response ratios were 0.78 and 0.80, respectively. Knowledge of how blood and brain concentrations of TRI relate to behavioral changes, coupled with a validated physiologically-based pharmacokinetic model for TRI, could be used to accurately predict TRI-induced behavioral alterations.

An operant testing system has been established in the inhalation toxicology laboratory. A modular test cage for rodents (Coulbourn Instruments) has been adapted for use in inhalation exposure chambers. Output from the cage is analyzed by a modular behavioral analysis instrument panel (Coulbourn Instruments), with component units selected for the operant tests envisioned for these studies. The performance schedules are applied with a software program (Cosmos, Coulbourn Instruments), and all data is stored on an IBM-compatible 386 computer. Different schedules and conditions are being investigated in order to optimize the results that can be obtained with each test in each species. The efficacy of the neurobehavioral testing protocols have been evaluated by the following criteria: intrasubject variability on repeated testing; intersubject variability; adaptability for use with both species; technical feasibility; rapidity with which repetitive testing can be conducted (on each subject); objectivity; sensitivity; quantitativeness (i.e., ability to accurately reflect the degree of CNS dysfunction elicited over time by a range of vapor concentrations). Analysis of the data from the operant studies entailed calculating the response and reinforcement rates from the test animals during or after halocarbon exposure as a percentage of each subject's mean control values prior to exposure.

Specially tooled operant boxes were positioned inside dynamic flow inhalation chambers. Male Sprague-Dawley rats (275-325 g) were food restricted (10 g/day) and trained to elicit presses for evaporated milk presentation (0.08 ml) on a VI 30 schedule for 2 hr. Once response rates were stable, rats were exposed to clean air for 20 min followed by either 500, 1000, 2000, 3500, or 5000 ppm TRI for 100 min. The number of lever presses in each 5 min interval of the operant session was computer recorded and TRI concentrations were monitored with a Miran 1B2 infrared spectrophotometer. Response ratios were calculated by dividing the number of responses during exposure by the average number of responses during the three control sessions immediately preceding exposure. Alternatively, changes in operant response rates were quantified by calculating the difference between the areas under the predicted and observed cumulative response curves during TRI exposure. Cumulative response curves were predicted by linear extrapolation of response rates during the first 20 min of each exposure session.

Male, Sprague-Dawley rats (275-325 g) were food restricted (10 g/day) and gavaged with evaporated milk (10 ml/day) to provide a diet comparable to that of the animals used in the behavioral study. Rats were exposed to 1000, 2000, 3500, or 5000 ppm TRI for either 10, 20, 40, 60, 80, or 100 min at which time they were sacrificed by cervical dislocation. Samples of blood, brain, liver, kidney, fat, muscle, heart, spleen, GI, and muscle were quickly removed and immediately placed in chilled scintillation vials containing 8 ml of isoctane and 2 ml of saline. Tissues were homogenized, vortexed, and centrifuged. Blood samples and aliquots of the tissue supernatant were placed in 20 ml headspace vials and analyzed with a headspace sampler unit of a Perkin Elmer Model 8500 gas chromatograph. The TRI concentrations in blood and tissues were calculated from a standard curve and corrected for the percent recovery characteristic of blood and tissue samples.

Operant responding was unaffected by inhalation of 500 or 1000 ppm TRI, whereas 2000, 3500, and 5000 ppm TRI decreased responding in a dose-dependent manner (Figure 1, Section H of the Appendix). The time of onset and the magnitude of the decrease were dose-dependent. The area under the curve (AUC) method of quantifying changes in operant response rates, while resulting in a similar dose-response curve to that generated by a

traditional method did not reflect the rate changes induced by inhalation of 2000 ppm TRI (Figure 2, Section H of the Appendix).

The uptake and disposition of TRI in blood and tissues were dose-dependent (Figure 3 and Figure 4). The highest concentrations of TRI were in the fat, followed by the liver and the brain. The relationships between blood and brain concentrations of TRI and mean operant response ratios were curvilinear, with correlation coefficients of 0.78 and 0.80, respectively (Figure 5). Behavioral deficits may, therefore, be reasonably predicted by blood and brain concentrations of TRI.

XIX. COMPARISON OF TRI TOXICOKINETICS WITH NEUROBEHAVIORAL TOXICITY DURING INHALATION EXPOSURE

In the studies on the CNS effects of VOCs, schedule-controlled behavior has been frequently employed as a quantitative endpoint to measure neurobehavioral toxicity. The inhalation of TRI by test animals has been associated with reductions in the responding rates of operant behavior using various reinforcing schedules. However, there is a surprising paucity of data relating the disposition of VOCs in the blood and brain (the target organ) and these neurobehavioral effects. This data is of particular important to the testing of the hypothesis in this grant of evaluating relative toxic effects between species with equivalent VOC concentration in the target organ. Therefore, correlated studies were conducted to compare neurobehavioral effects in mice inhaling TRI with the blood and brain disposition of the compound.

These studies are included in a paper that is currently in press in the journal *Toxicology*. The galley proofs for this paper have been included as Appendix G, and the reference is as follows:

You, L., Muralidhara, S., and Dallas, C.E. "Comparisons between operant responding and 1,1,1-trichloroethane toxicokinetics in mouse blood and brain." *Toxicology*, 93: 151-163 (1994).

The effect of 1,1,1-trichloroethane (TRI) inhalation on operant response was evaluated in relation to the concentration of TRI in blood and brain tissue in mice during exposure. Male CD-1 mice were trained to lever-press for an evaporated milk reinforcer on a variable interval (VI 60) schedule for 2 h. Trained mice were then exposed to either 3500 or 5000 ppm TRI for up to 100 min, and the changes in the schedule-controlled performance were measured. Additional groups of mice were exposed under the same conditions as those used in the behavioral study and sacrificed at various times during exposure, and the blood and brain samples were collected and subsequently analyzed for TRI content by headspace gas chromatography. Uptake of TRI into blood and brain was rapid, with near steady-state levels reached after approximately 40-60 min of exposure. Inhalation of 5000 ppm, but not 3500 ppm TRI was seen to cause inhibition of operant response, starting ~30 min following the initiation of inhalation exposure and beginning to recover after 80 min of exposure. The threshold concentrations for the maximal behavioral inhibition were ~110 µg/g and 130 µg/ml in mouse brain and blood, respectively. It appears that in addition to TRI concentrations in

blood and brain tissue, the time it takes to reach the apparent threshold TRI concentration was also a determinant for the onset of TRI neurobehavioral depression.

In order to appropriately correlate the toxicokinetics of these agents with the neurotoxicity response, one additional factor of potential importance to these lipophilic chemicals had to be addressed. It has been recognized that diet is one of the factors that can alter the pharmacokinetics and toxicity of a compound. The pattern, quantity, and content of dietary intake is able to change the responsiveness of a biological system to a toxicant, as well as to influence the bioavailability of the chemical in the system. However, the consequence of high milk intake on VOC pharmacokinetics that would occur during the behavioral sessions of neurobehavioral toxicity studies has not been examined previously. Since an effect on the pharmacokinetics of a toxicant often results in an alteration in toxicity, this study was undertaken to determine the potential impact of high milk intake during operant training on the pharmacokinetics of inhaled VOCs.

The paper on this study has been published in the journal *Drug and Chemical Toxicology*. A copy of this paper has been included as Appendix H, and the reference is as follows:

You, L., Muralidhara, S., and Dallas, C.E. "The pharmacokinetics of inhaled 1,1,1-trichloroethane following high milk intake in mice." *Drug and Chemical Toxicology*, 17: 479-498 (1994).

In the evaluation of lipophilic halocarbons for neurobehavioral toxicity in operant testing, animals often receive large amounts of milk as a behavioral reinforcer over time. If this increase of fat in the diet sufficiently impacted the lipid depots of the animal, the pharmacokinetics of lipophilic test compounds might be significantly affected and thus obscure the accompanying neurobehavioral effects. The effects of milk intake, comparable to what was consumed as behavioral reinforcer during operant behavioral sessions, on the pharmacokinetics of inhaled 1,1,1-trichloroethane (TRI) were therefore examined in the blood and nine organ tissues of mice. Male CD-1 mice were food restricted so that their body weights would be reduced to and maintained at 80% of their original, and received a single gavage dose of 1.0 ml evaporated milk daily for three weeks. A control group with similar food restrictions was dosed with the same volume of water. Inhalation exposures to 3500 ppm TRI for 100 minutes were conducted at the end of the treatment period. Blood and nine organ tissues were sampled at a series of time points, and their TRI contents were analyzed by headspace gas chromatography. The uptake of TRI was rapid, with near steady state approached in blood and most tissues after 40-60 minutes of exposure. All of the tissues except fat had similar TRI time-concentration profiles, while TRI concentrations in fat tissue were about 20-30 times higher than in other tissues. There was no statistically significant difference in the tissue concentrations between the milk-dosed group and water-dosed group at all of the time points for all tissues measured. Therefore, it appears unlikely that this level of milk intake as a reinforcer in behavioral studies will affect the results of operant testing evaluations by altering the pharmacokinetics of lipophilic halocarbons such as TRI.

Both of these studies in Section VII were presented at the most recent meeting of the Society of Toxicology in Dallas, TX (March, 1994). The reference for the abstract of this presentation is as follows:

You, L., Muralidhara, S., and Dallas, C.E. "Neurobehavioral effects of 1,1,1-trichloroethane and its relationship with brain concentration in mice," *Toxicologist* 14: 352, 1994.

XX. CORRELATION OF PCE TOXICOKINETICS AND BEHAVIORAL EFFECTS FOLLOWING ORAL EXPOSURE IN RATS

The majority of reports of PCE-induced human toxicity has focused upon the neurological effects among the occupationally or experimentally exposed. Acute exposure in the 100 to 200 ppm range have typically resulted in reversible mood and behavioral changes and impaired coordination. Major EEG changes suggestive of cerebral cortical depression have been found among volunteers repeatedly exposed to 100 ppm. Subchronic exposure to even lower PCE concentrations has reportedly caused memory loss and insomnia as well as perceptual, attention, and intellectual deficits.

Acute neurological effects in humans after ingesting PCE appear to parallel those seen after inhalation. The oral administration of PCE to hundreds of thousands of patients as an anthelmintic agent at doses of 60 to 85 mg/kg (assuming a 70 kg body weight with doses of 4.2 to 6 g) typically produced inebriation, perceptual distortion, and exhilaration. The accidental ingestion of 600 to 800 mg/kg (assuming a 20 kg body weight with a dose of 12 to 16 g) by a 6-year-old boy was followed by drowsiness, vertigo, agitation, and hallucinations, prior to somnolence and coma.

The neurological effects of PCE exposure may be expressed as subtle behavioral changes that jeopardize one's state of well-being. It is important that such changes be assessed in an animal model in a valid, quantitative manner. Tests employing schedule-controlled operant behavior (SCOB) have demonstrated chemically related behavioral effects prior to irreversible neuropathological changes for a number of solvents, including toluene, trichloroethylene, and trichloroethane. While PCE's effects on open-field behavior motor activity and neuromuscular ability have been investigated, PCE's effect on the SCOB of laboratory animals has not been reported.

It has been suggested that the biological effects of solvents may be more closely related to blood or target tissue (i.e., brain) concentrations than administered dose (references). Unfortunately, efforts to integrate the pharmacokinetics of solvents with neurobehavioral effects have been few. It has been demonstrated that brain and blood toluene levels were highly correlated with the degree of CNS depression in mice, as measured in tests of unconditioned performance and reflexes. More recently, it has been shown that shock avoidance performance decrements were closely related to trichloroethylene levels in the blood of rats. It has also been reported that a correlation exists between blood trichloroethane levels and performance decrements in human volunteers. To date, however, no parallel investigations of the pharmacokinetics and neurobehavioral effects of PCE have been reported. Thus, the present study was designed to evaluate the relationship between the pharmacokinetic distribution of orally administered PCE and its effects on SCOB of rats.

This paper has been published in the journal *Toxicology and Environmental Health*. The paper is included as section I of the Appendix, and the reference is as follows:

Warren, D.A., Reigle, T.G., Muralidhara, S., and Dallas, C.E. "Schedule-controlled operant behavior of rats following oral administration of perchloroethylene: time-course and relationship to blood and brain levels." *Toxicology and Environmental Health*, 47: 101-118, 1996.

1,1,2,2-tetrachloroethylene (PCE) of 99% + purity was obtained from Aldrich Chemical Company, Inc. Burdick and Jackson Brand, High Purity Solvent iso-octane was obtained from Baxter Healthcare Corporation. Chemically naive, male Sprague-Dawley rats weighing 300 to 350 g were used in all experiments. Rats were housed two per cage in suspended wire bottom cages (36 x 20 x 20 cm) in a temperature- (22°C) and humidity- (45%) controlled room with a 12-hr light-dark cycle (light: 0700-1900 hr; dark: 1900-0700 hr). Rats were allowed to acclimate for a minimum of 7 days prior to use, during which time food and tap water were provided ad libitum. All experiments were conducted during the light cycle.

Twelve rats were transferred to individual polypropylene cages (48 x 25 x 20 cm) with corn cobb bedding and stainless steel wire lids. Rats were food restricted (10 ± 0.25 g/day) for 72 hr prior to being surgically implanted with an indwelling carotid artery cannula. Rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg body wt. of a mixture of ketamine HCL (100 mg/ml): acepromazine maleate (10 mg/ml):xylazine HCl (20 mg/ml) in the proportion 3:2:1 (v:v:v). The cannula exited the skin at the nape of the neck and was protected from manipulation by surgical tape. The cannulated animals were placed in individual polypropylene cages (30 x 20 x 15 cm) with wire mesh tops for surgical recovery and subsequent blood sampling. After an overnight recovery period, either 160 or 480 mg/kg body wt. PCE was administered as an aqueous Alkamuls® emulsion by bolus gavage in a total volume of 3 ml/kg body wt. Ten (160 mg/kg body wt.) or 20% (480 mg/kg body wt.) Alkamuls EL-620 was used to prepare stable emulsions of PCE in 0.9% saline. The actual concentration of PCE in each dosage formulation was determined from a standard curve made by diluting various amounts of PCE in isoctane for analysis by headspace gas chromatography (GC). Following dosing, blood samples were withdrawn from the arterial cannulas of the unrestrained and unanesthetized animals via a three-way stopcock by a 1 ml syringe. Serial blood samples (2 to 75 μ l, depending upon the anticipated blood concentration) were taken at time intervals of 1 min to 24 hr for up to 4 days after dosing. Blood samples were quickly transferred to 8 ml headspace vials, capped immediately with teflon-lined latex rubber septa in aluminum seals, and crimped tightly. Some blood samples required dilution with ice-cold saline in order that they could be analyzed within the linear range of the electron capture detector of the GC. As necessary, blood withdrawal was followed by a heparin flush to maintain cannula patency. Food was withheld during surgical recovery (approximately 18 hr) but was available ad libitum during blood sampling.

For the determination of PCE concentrations in tissues, rats were transferred to individual polypropylene cages (48 x 25 x 20 cm), food restricted (10 ± 0.25 g/day) for 72 hr, and subsequently fasted for 18 hr prior to being dosed as described for blood sampling. Groups of six rats were sacrificed by cervical dislocation followed by decapitation at 1, 6, 15, 30, 40, 50, 60, and 90 min after dosing. Approximately 0.5 to 1.0 g samples of brain, liver, perirenal fat, and skeletal muscle were excised with 2.5 to 3 min from each animal and immediately placed into chilled scintillation vials containing 2 ml of 0.9% saline and 8 ml of isoctane. Tissues were homogenized as quickly as possible (5 to 15 seconds) with an Ultra-Turrax® homogenizer to minimize volatilization of PCE prior to being vortex-mixed for 30

seconds. The homogenates were then centrifuged at 2500 x g for 10 min at 4°C in the capped scintillation vials. An aliquot of the isoctane layer (5 to 20 µl) was either transferred directly to a 20 ml headspace vial. The vials were capped immediately with teflon-lined latex rubber septa in aluminum seals and crimped tightly.

A Sigma Model 300 GC equipped with a HS6 headspace sampler and an electron capture detector (ECD) was used for the analysis of PCE in blood. Analyses were carried out using a stainless-steel column (182 x 0.317 cm) packed with 3% OV-17 (100-120 mesh). The GC operating conditions were: headspace sampler temperature, 80°C; injection port temperature, 200°C; column temperature, 90°C; ECD temperature, 360°C; flow rate for argon:methane (95:5) carrier gas, 60 ml/min. For PCE analysis of isoctane tissue extracts, a Perkin-Elmer Model 8500 GC with a HS-101 headspace autosampler and ECD was employed under the same conditions as those previously listed. All sample vials were heated thermostatically in one of the two headspace sampler units, pressurized with the carrier gas, and a pre-set volume of volatilized isoctane and PCE was injected into the GC column. PCE concentrations were calculated from daily standard curves made by diluting various amounts of PCE in isoctane for GC analysis and corrected for the percent recovery characteristic of blood and tissue samples. Percent recovery of PCE was determined by injecting solutions of PCE in isoctane into samples of blood and each of the four tissue types with a Hamilton gas-tight syringe. The blood and tissues were then homogenized in saline/isoctane as previously described and aliquots of the isoctane analyzed for PCE content. Percent recoveries ranged from 86% for fat to 92% for muscle. The limit of detection for PCE was approximately 1 ng.

Fourteen rats were transferred to individual polypropylene cages (48 x 25 x 20 cm) with corn cobb bedding and stainless steel wire lids. Rats were food-restricted during a period in which they were trained to lever-press for undiluted evaporated milk presentation (0.05 ml for 7 seconds) on a fixed ratio-40 schedule of reinforcement. Rats were initially hand shaped in daily 30 min sessions. Once rats learned to respond independently, the ratio of responses to reinforcers was gradually increased to 40 and the session length extended to 90 min. Rats were allowed to respond in daily 90 min sessions spaced 24 hr apart until their response rates stabilized, a process requiring 10 to 15 days. The criterion for stable behavior was three successive sessions in which the number of responses per second varied by less than 10%.

Upon the completion of each operant session, rats were returned to their home cage and given 10 ± 0.25 g of food which was promptly eaten. Since 24 hr separated each operant session, this feeding regimen resulted in a fasting period of approximately the same duration as that employed in the study of PCE's pharmacokinetics. Once rats exhibited stable behavior, they were gavaged with either a 10 or 20% aqueous Alkamuls emulsion vehicle (3 ml/kg body wt.), immediately placed in a modular test cage, and monitored for operant behavior for 30 min. Twenty-four hr later, rats were dosed with either 160 or 480 mg/kg body wt. PCE in the appropriate vehicle (3 ml/kg body wt.), and their behavior monitored again. The behavior of six and eight rats was monitored after administration of the low and high doses, respectively.

Operant sessions took place in a modular test cage for rodents equipped with a response lever, a liquid delivery trough and dipper, and a stimulus light above the delivery trough that remained lit during the availability of the milk reinforcer. The modular test cage was placed inside a 1.0 M³ Rochester-type dynamic flow inhalation chamber that served to

isolate animals from extraneous external stimuli. The test cage was interfaced via LabLinc with an IBM compatible 386 computer running COSMOS software that applied the operant performance schedules and recorded the number of responses and reinforcers in each 5-min interval of the operant sessions.

RSTRIP (Version 3.1) was used to fit PCE blood concentration versus time profiles to polyexponential equations and for calculating AUC, $t\frac{1}{2}$, and Cmax. Tests for differences in the concentrations of PCE in blood and tissues were made with two sample t-tests. The baseline response of each rat was calculated as the mean number of responses in each 5 min interval of the three operant sessions used to meet the stability criterion. Trends in response(s) were examined by fitting a straight line to the baseline response of each rat and calculating a t-statistic based on the mean \pm SD or regression coefficients (slopes) for comparison to critical t values. Response ratios (vehicle/baseline, (PCE + vehicle)/baseline, and (PCE + vehicle)/vehicle) for each of the eighteen 5 min intervals in the operant sessions were calculated so that each rat served as his own control. These ratios were subjected to a mixed model repeated measures analysis of variance (RMANOV) with fixed factors of time and treatment, and a random factor of rat nested within treatment. In the event that time effects failed to satisfy multisample sphericity, test statistics of time effect and time x treatment interaction were modified using the Geisser-Greenhouse adjustment factor. Despite non-significant time x treatment interactions, two sample t-tests were used to determine the 5 min intervals during which response ratios differed between dose groups. Paired t-tests within each dose group were used to determine the intervals during which response ratios significantly differed from one another. Due to equipment failure, response data from portions of two operant sessions used to meet the stability criterion were lost. These data were presumed to be equal to that in the last 5 min interval successfully recorded. Following gavage with 480 mg/kg body wt. PCE< two rats failed to respond in all but two intervals. Exclusion of these non-responders did not change the conclusions drawn from variance analysis, but did slightly modify P values. Unless otherwise noted, data analysis was exclusive of the non-responders. The minimum level of significance was set at $p \leq 0.05$ for all tests.

The blood concentration versus time profiles for rats following gavage at both doses (160 and 480 mg/kg) are presented in Figs. 1 and 2. The long elimination half-life ($t\frac{1}{2}$) of PCE relative to that of most volatile halocarbons was demonstrated by its presence in blood for several days after dosing. The pharmacokinetic parameter estimates for each treatment group are summarized in Table 1. The differences in AUC and Cmax between the two doses were nearly proportional to the difference in PCE dose. A 3-fold increase in dose resulted in AUC and Cmax values that were 2.7 and 3.6-fold higher, respectively. Blood $t\frac{1}{2}$ did not differ with dose.

The blood and brain concentration versus time profile during the 90 min immediately following dosing are presented in Figs. 3 and 4, respectively. This 90-min post dosing period corresponds to the time course over which SCOB was monitored. PCE was rapidly absorbed from the gastrointestinal tract as evidenced by its presence in blood as early as 1 min after dosing. As might be anticipated for a highly perfused tissue, PCE was also present in the brain at this time. Following a 10 to 15 min phase of very rapid PCE uptake, blood and brain concentrations slowly increased as they approached a steady-state equilibrium. Absolute maximum blood concentrations were not reached until 30 and 90 min after administration of

the low and high doses, respectively. Following low dose gavage, the maximum brain concentration occurred at 60 min, but still increasing 90 min after administration of 480 mg/kg PCE.

In both the blood and brain, the ratio of PCE concentrations resulting from the two doses was greatest at 1 min (9.5 in blood and 3.0 in brain). Blood PCE concentrations significantly differed with dose at all sampling times during the 90 min immediately following dosing ($p \leq 0.0018$). Despite a 3-fold difference in mean brain PCE concentrations at 1 min, the difference was not statistically significant ($p = 0.072$) due to variability within the high dose group. Brain concentrations at 6, 15, and 20 min also were not significantly different across doses ($p = 0.1080$, $p = 0.2139$, and $p = 0.4488$, respectively), but were so at all subsequent sampling times ($p \leq 0.0068$).

Tissue dose time courses for PCE were also determined in fat, liver, and muscle (Figs. 5 and 6). The rate of blood perfusion and lipid content of these tissues had a significant impact on organ deposition in the rat. Based upon relative tissue concentrations at 1 and 6 min, the well perfused liver accumulated PCE at the highest rate, followed by the fat and muscle. Based on concentrations at 1 and 6 min, PCE accumulation by the brain was most similar to that of the fat, not the liver as might be predicted. Prior to 30 min post dosing, tissue concentrations rarely differed with dose. At 90 min post dosing, at which time behavioral monitoring was discontinued, PCE concentrations were greatest in the fat, followed by the liver, brain, and muscle.

The baseline response rates of rats in the low and high dose treatment groups ranged from 1.04 to 3.04 (mean \pm SD, 1.88 ± 0.75) and from 0.99 to 2.62 (1.63 ± 0.58) responses per second, respectively (Figs. 7 and 8). Neither treatment group exhibited a significant trend in baseline responding, i.e., baseline response rates did not have a tendency to significantly increase or decrease over the course of the operant sessions. However, straight lines fit to the baseline responses of eleven of the fourteen rats had negative slope values indicating slight decreases in response rates did occur over time.

The repeated measures analysis of variance on vehicle/baseline (V/B) ratios indicated no significant time \times treatment interaction ($p = 0.3349$), but significant treatment ($p = 0.0159$) and time ($p = 0.0254$) effects. Despite the high dose group having a higher mean V/B ratio during sixteen of the eighteen operant session intervals, group differences were significant only between 5-10 ($p = 0.0341$) and 10-15 ($p = 0.0184$) min. Response rates of the treatment groups averaged 91.6 ± 5.2 and $111.0 \pm 18.2\%$ of baseline after administration of the 10 and 20% aqueous emulsion vehicles, respectively (Figs. 9 and 10). Mean V/B ratios of the low dose group were less than one during fourteen session intervals. In contrast, mean V/B ratios of the high dose group were greater than one in fifteen intervals. These deviations from unity by the low dose group were significant only between 0-5, 5-10, 10-15, 30-35, and 40-45 min ($p = 0.0099$, 0.0107 , 0.0197 , 0.0252 , and 0.0297 , respectively), while none of the mean V/B ratios of the high dose group significantly differed from one.

Variance analysis of (PCE + vehicle)/baseline (PV/B) ratios indicated no significant time \times treatment interaction ($p = 0.2794$) or treatment effect ($p = 0.1870$), but a significant time effect ($p = 0.005$) (Figs. 11 and 12). Despite the low dose group having a higher PV/B ratio

during fourteen of the eighteen session intervals, group differences were not significant during any interval. Response rates of the treatment groups averaged 99.4 ± 9.8 and $83.6 \pm 16.0\%$ of baseline after administration of the low and high PCE doses, respectively. The inclusion of data on the two-non-responders reduced the mean response rate of the high dose group to $62.9 \pm 40.6\%$ of baseline. Mean PV/B ratios of the low dose group were less than one in eight intervals, including five of the first seven (Fig. 13). Mean PV/B ratios of the high dose group were less than one in thirteen intervals including eleven of the first twelve (Fig. 14). These deviations from unity by the low dose group were significant only between 0-5, 50-55, and 80-85 min ($p = 0.0329, 0.0221$, and 0.0396 , respectively), while V/B ratios of the high dose group significantly differed from one at 0-5 ($p = 0.0002$) and 10-15 ($p = 0.0220$) min.

When (PCE + vehicle)/vehicle (PV/V) ratios were submitted to variance analysis, there was no significant time x treatment interaction ($p = 0.4153$), but a significant treatment ($p = 0.0012$) and time ($p = 0.0230$) effect. Even though the low dose group had a higher mean PV/V ratio during all of the session intervals, group differences were only significant between 0-5 ($p = 0.0357$), 10-15 ($p = 0.0051$), and 15-20 ($p = 0.0163$) min. Response rates of the treatment groups averaged 107.2 ± 13.3 and $75.8 \pm 17.7\%$ of vehicle after administration of the low and high PCE doses, respectively. The inclusion of data on the two non-responders reduced the mean response rate of the high dose group to $57.0 \pm 37.7\%$ of vehicle. Mean PV/V ratios of the low dose group were greater than one in seventeen intervals. On the otherhand, mean PV/V ratios for the high dose group were less than one in seventeen intervals. None of the deviations form unity were significant in the low dose group, while response ratios during the first four intervals ($p = 0.0018, 0.0512, 0.0135$, and 0.0376 , respectively) were significantly different form one in the high dose group. Inclusion of data from the two non-responders in the high dose group resulted in significant deviations from one in ten intervals, including none of the first ten.

XXI. DETERMINATION OF THE BLOOD AND TARGET TISSUE PHARMACOKINETICS OF INHALED 1,1,2-TRICHLOROETHYLENE (TCE) AND ITS MAJOR METABOLITES IN MICE

Studies with B6C3F1 mice have linked two metabolites of TCE, trichloroacetate (TCA) and dichloroacetate (DCA), with liver cancer. TCE is metabolized to a transient epoxide which rapidly undergoes intramolecular rearrangement to form trichloroacetylaldehyde, which is either oxidized to TCA or reduced to trichloroethanol. DCA is formed via a dechlorination reaction. The human TCE metabolic profile is qualitatively, although not quantitatively, the same as that of test animals. For the purpose of risk assessment, the liver and lung of the B6C3F1 mouse represent the most sensitive organs in TCE cancer bioassays. Therefore, a comprehensive PBPK model for TCE for use in cancer risk assessment should not only predict the concentration time course of TCE in these organs, but of its active metabolites as well. Although no such model currently exists, a massive research effort is underway with this goal in mind. Experimental data for validation purposes will thus be needed on TCE and its metabolites in blood, liver, and lung. Quantification of the parent compound in fat is also important since this tissue is the primary repository for TCE, and thus a critical determinant of TCE's pharmacokinetics. As previously mentioned, it is advantageous to have quantitative toxicity data with which to correlate blood and tissue concentrations. The blood and tissue

concentration time course determination thus employed B6C3F1 mice subjected to an exposure scenario for which positive cancer bioassay data exist.

Physiologically-based pharmacokinetic models have recently become an important tool in cancer risk assessments for several chemicals, including TCE. It is absolutely necessary to assess the precision of PBPK models by comparing model predictions to experimentally-determined blood and tissue concentration time courses distinct from those on which the models are based. Unfortunately, there exist very few comprehensive experimental data sets for TCE model validation. Further progress in applying PBPK models to TCE risk assessment is therefore dependent upon obtaining blood and tissue concentration time course data through direct measurement studies. The opportunity to perform such a study in the Toxic Hazards Division at Wright-Patterson Air Force Base recently presented itself. Collaboration with this DOD laboratory will provide the ability to extend the modelling efforts to the metabolites as well as the parent compound.

Experimentally-naive, male B6C3F1 mice (28-32 g) were acclimated on a 12-hr light-dark cycle in a temperature- and humidity-controlled room for a minimum of 7 days prior to use. Tap water and commercial rodent chow were provided ad libitum. This project was conducted in the Toxic Hazards Division, Armstrong Laboratory, Wright-Patterson Air Force Base, Dayton, OH.

Sixteen mice were exposed to 600 ppm TCE to determine blood and tissue concentrations of the parent compound and its metabolites during and following exposure. A relatively new nose-only exposure system was adapted for this purpose (Fig. 1). The nose-only system can support fifty-two animal holding tubes (350 ml each), only seventeen of which were utilized. Sixteen tubes held mice, one tube was connected to a GC to monitor the TCE concentration, and the remaining tubes were closed off with rubber stoppers. Trichloroethylene vapor was generated using a 250 ml gas washing bubbler filled with TCE that was maintained at 0°C with a slush ice bath. The TCE concentration was monitored using a Varian 3400 GC with a loop injection system and a flame ionization detector. The target TCE concentration of 600 ppm was maintained by very slight adjustments of the exhaust flow.

Just prior to exposure, sixteen mice were loaded into their respective tubes. When a sampling point along the time course arrived, a tube (with mouse inside) was removed from the exposure system and the hole stoppered without affecting the TCE concentration in the other tubes. One tube was removed at the following times during exposure: 15 min, 30 min, 1 hr, 3 hr, 5 hr, and 7 hr. The mice in these tubes were immediately sacrificed for blood and tissue collection. Immediately following removal of the tube at 7 hr, all remaining tubes were removed (i.e., the maximum duration of exposure was 7 hr). The mice from these tubes were placed in individual holding cages with access to food and water ad libitum. A mouse was removed from its holding cage and sacrificed for blood and tissue collection at each of the following times post exposure: 5 min, 15 min, 30 min, 45 min, 1.5 hr, 3 hr, 5 hr, 7 hr, 9 hr, and 18 hr. All mice were sacrificed by CO₂ asphyxiation, blood was withdrawn from the inferior vena cava, and liver, lungs, and perirenal fat were excised. The blood and liver samples were divided into two parts, one for parent compound analysis and the other for metabolite analysis. The lung and fat tissue volumes were insufficient to divide and were only used to determine TCE concentrations. Blood and tissue levels of TCE were determined by

the method of Chen *et al.* (1993). For metabolite analysis, TCE and DCA were derivitized to their methyl esters and 2,2-dichloropropionic acid was used as an internal standard. The methyl esters and 2,2,2-trichloroethanol were analyzed by ECD-GC following liquid injection. The above scenario was repeated five times in order that the blood and tissue concentration time course would accurately reflect the variation between animals.

Thus far, only TCE data have been tabulated. The TCE concentration time courses for blood, liver, lungs and fat are shown in Fig. 2-5, respectively. In addition to the experimentally-measured blood and tissue concentrations, the figures also contain PBPK model predictions (solid lines). In all cases, the model predicts a more rapid rise in TCE concentrations than is reflected by the measured values. With the exception of the liver, the model overpredicts the maximum TCE concentration, although the rate of TCE elimination is predicted fairly accurately. The model also predicts that blood and tissue concentrations will reach a steady-state equilibrium which is not reflected in the experimental data. The reason for the discrepancies between model predictions and measured values are unknown. A previous attempt to model TCE pharmacokinetics in this concentration range in the B6C3F1 mouse was also unsuccessful. The validity of the experimental data will be tested through direct and systematic replication at WPAFB in the near future.

XXII. NEUROBEHAVIORAL CHANGES DURING TRI INHALATION IN RELATION TO PHARMACOKINETICS

The central nervous system is the principal target of 1,1,1-trichloroethane (TRI), and several studies of this volatile solvent have demonstrated effects on learned animal behaviors. There have been no attempts, however, to quantitatively relate such effects to blood or target organ (i.e., brain) solvent concentrations. Therefore, Sprague-Dawley rats trained to lever-press for evaporated milk on a variable interval-30 second reinforcement schedule were placed in an operant test cage and exposed to clean air for 20 minutes, followed by a single concentration of TRI vapor (500-5000 ppm) for 100 minutes. Additional rats were exposed to equivalent TRI concentrations for 10, 20, 40, 60, 80 or 100 minutes to determine blood and brain concentration versus time profiles. Inhalation of 1000 ppm slightly increased operant response rates, whereas 2000, 3500 and 5000 ppm decreased operant response rates in a concentration- and time-dependent manner. Accumulation of TRI in blood and brain was rapid and concentration-dependent, with the brain concentration roughly twice that of blood. Plots of blood and brain TRI concentrations against operant performance showed responding in excess of control rates at low concentrations, and decreasing response rates as concentrations increased. Linear regression analyses indicated that blood and brain concentrations were strongly correlated with, and equally predictive of, operant performance. Neurobehavioral toxicity in laboratory animals, as measured by changes in operant performance, can therefore be quantitatively related to internal measures of TRI exposure to enhance its predictive value for human risk assessment.

This paper is in the process of being submitted to a peer-reviewed journal, *Neurotoxicology and Teratology*. The manuscript is included as section J in the Appendix. The reference for the manuscript is as follows:

Warren, D.A., Reigle, T.G., Christmus, W.H., Muralidhara, S., and Dallas, C.E. "Schedule-controlled operant behavior of rats during 1,1,1-trichloroethane inhalation: Relationship to blood and brain solvent concentrations." To be submitted to *Neurotoxicology and Teratology*.

XXIII. NEUROBEHAVIORAL EFFECTS FOLLOWING SINGLE AND REPEATED INHALATION EXPOSURES TO TRI

A design feature of most dose-response studies involving schedule-controlled operant behavior is the repeated administration of different doses of the test substance to the same experimental animal. Repeated dosing raises the question of whether or not an animal's initial exposure to a chemical agent alters its behavioral response to subsequent exposures. To address this question, a dose-response curve for the effect of inhaled 1,1,1-trichloroethane (TRI) on the rate of lever-pressing for milk delivery was generated with repeatedly exposed rats (i.e., a within-subject design) and compared to dose-response data obtained from rats receiving a single inhalation exposure to TRI (i.e., a between-group design). Relative to that generated with singly exposed rats, the dose-response curve generated by repeated exposure was shifted to the left. This suggests that the behavioral effects of rate-decreasing concentrations of TRI are augmented by previous exposures. This residual effect is apparently not due to the accumulation of pharmacologically active substances or to the development of an aversion to responding, since TRI is rapidly eliminated following exposure and solvent-free responding was unaffected 24 hours post exposure. Instead, the results of this study support the well established belief that an animal's response to a drug or chemical agent can be modified by its prior behavioral and exposure history. Thus, comparisons of single and repeated exposures are essential for fully accurate interpretations of the behavioral consequences of solvent exposure.

This paper is in the process of being submitted to a peer-reviewed journal, the *Journal of the American College of Toxicology*. The manuscript is included as section K in the Appendix. The reference for the manuscript is as follows:

Warren, D.A., Reigle, T.G., and Dallas, C.E. "Dose-response curves for the effect of 1,1,1-trichloroethane on the operant behavior of singly and repeatedly exposed rats." To be submitted to *Journal of the American College of Toxicology*.

XXIV. RELATIONSHIP OF THE PHARMACOKINETICS OF INHALED TRI TO NEUROBEHAVIORAL EFFECTS IN MICE

Despite the central nervous system (CNS) being a target of virtually all solvents, there have been few studies of solvent effect on unlearned animal behaviors. Little is known about the relationship of exposure concentration to behavioral effect, and quantitative data relating the toxicologically important target organ (i.e., brain) dose to behavioral effect are almost non-existent. To examine the relationships of blood and brain concentrations of 1,1,1-trichloroethane (TRI) to locomotor activity, mice were exposed to TRI (500-14,000 ppm) in static inhalation chambers for 30 minutes, during which locomotor activity was measured.

Separate mice were exposed to the same concentrations for 6, 12, 18, 24 and 30 minutes to determine blood and brain concentration versus time profiles for TRI. The lowest TRI concentration studied (500 ppm) had no effect on activity, intermediate concentrations (1000-8000 ppm) increased activity immediately to levels that remained constant over time, and higher concentrations (10,000-14,000 ppm) produced biphasic effects, i.e., increases in activity followed by decreases. TRI concentrations in blood and brain approached steady-state equilibria very rapidly, demonstrated linear kinetics, and increased in direct proportion to one another. Locomotor activity increased monophasically (\approx 3.5 fold) as solvent concentrations increased from approximately 10-160 $\mu\text{g/g}$ brain and $\mu\text{g/ml}$ blood. As concentrations exceeded the upper limit of this range, the activity level declined and eventually fell below the control activity level at approximately 250 $\mu\text{g/g}$ brain and $\mu\text{g/ml}$ blood. The broad dose range employed demonstrated that TRI, like some classical CNS depressants, is capable of producing biphasic effects on behavior, supporting the hypothesis that selected solvents are members of the general class of CNS depressant drugs. By relating internal dose measures to locomotor activity, our understanding of the effects observed and their predictive value may be enhanced.

This paper is in the process of being submitted to a peer-reviewed journal, *Neurotoxicology and Teratology*. The manuscript is included as section L in the Appendix. The reference for the manuscript is as follows:

Warren, D.A., Bowen, S.E., Dallas, C.E., and Balster, R.L. "Biphasic effects of 1,1,1-trichloroethane on the locomotor activity of mice: Relationship to blood and brain solvent concentrations." To be submitted to *Neurotoxicology and Teratology*.

XXV. SUMMARY AND CONCLUSIONS, DISSERTATION OF ALAN WARREN, PH.D. (1995)

The extrapolation to humans of neurobehavioral toxicity data collected in experimental animals, in the absence of comparative pharmacokinetic analysis, can lead to erroneous conclusions. However, if the assumption is true that halocarbon concentrations in the central nervous system of one species are equally as toxic in another, the behavioral response of humans to various halocarbon exposures might be predicted from animal data where brain dose, or a suitable dose surrogate, is correlated with changes in behavior. At present, interspecies extrapolations of neurobehavioral toxicity data on the basis of brain dose are virtually non-existent, due to the paucity of data on halocarbon tissue kinetics, neurobehavioral toxicity, and their quantitative relationship.

Time-courses of blood and brain concentrations and neurobehavioral toxicity were determined for two halocarbon solvents in two rodent species. Based on these data, quantitative relationships, or the lack thereof, have been reported between the degree of neurobehavioral toxicity and internal measures of dose. In the case of orally-administered perchloroethylene (PCE), relationships between blood and brain concentrations and operant performance were not discernable, due in part to an acute adaptation of rats to PCE's response suppressing effect. For inhaled 1,1,1-trichloroethane (TRI), blood and brain concentrations were strongly correlated with the rate of operant responding in rats. Responding was slightly in excess of control rates at low concentrations, and decreased in

a linear fashion as blood and brain concentrations increased. A robust biphasic response was seen in the locomotor activity of mice exposed to TRI by inhalation. Locomotor activity increased monophasically as solvent concentrations increased to a threshold concentration, above which activity declined and eventually fell below the control level.

The blood and brain concentration time-course data are currently of value for the validation and refinement of physiologically-based pharmacokinetic (PBPK) models for animals. The behavioral data sets are important additions to the toxicology literature in that they include the first studies of PCE's effect on operant responding and TRI's effect on the operant responding of rats, and a vast expansion of the concentration range used in the one existing study of TRI's effect on locomotor activity. However, the behavioral and pharmacokinetic data will be particularly valuable when used together to validate PBPK models that can predict the tissue pharmacokinetic patterns of halocarbons in experimental animals, and thus the magnitude of neurobehavioral toxicity expected. Validation of a PBPK model using this data could be very useful once the model is scaled up to humans, allowing insight into what exposure levels may or may not produce behavioral effects in humans.

Physiological and anatomical differences between species can influence the pharmacokinetics of halocarbons considerably. As a result, equivalent exposures to different species may not result in equivalent doses to target tissues, including the CNS. Therefore, the extrapolation to humans of neurobehavioral toxicity data collected in experimental animals, in the absence of comparative pharmacokinetic analysis, can lead to erroneous conclusions. However, if the assumption is true that halocarbon concentrations in the CNS of one species are equally as toxic in another, the behavioral response of humans to various halocarbon exposures might be predicted from animal data where brain dose is correlated with changes in behavior. Of potential use in this approach to interspecies extrapolation are physiologically-based pharmacokinetic (PBPK) models that can accurately predict tissue halocarbon concentrations in humans over time, under a variety of exposure conditions. At present, interspecies extrapolations of neurobehavioral toxicity data on the basis of brain dose are virtually non-existent, due to the paucity of data on halocarbon tissue kinetics, neurobehavioral toxicity, and their quantitative relationship.

Using high quantitative behavioral measures and a sensitive analytical method, time-courses of blood and brain concentration and neurobehavioral toxicity were determined for two halocarbon solvents in two rodent species. Based on these data, quantitative relationships, or the lack thereof, have been reported between the degree of neurobehavioral toxicity and internal measures of dose. In the case of orally-administered PCE, relationships between blood and brain concentrations and operant performance were not discernable, due in part to an acute adaptation of rats of PCE's response suppressing effect. For inhaled TRI, blood and brain concentrations were strongly correlated with the rate of operant responding in rats. Responding was slightly in excess of control rates at low concentrations, and decreased in a linear fashion as blood and brain concentrations increased. A robust biphasic response was seen in the locomotor activity of mice exposed to TRI by inhalation. Locomotor activity increased monophasically as solvent concentrations increased to a threshold concentrate, above which activity declined and eventually fell below the control level.

Considering the hypothesized mechanism by which solvents affect the CNS, brain concentration would appear to be a logical dose metric to correlate with behavioral toxicity.

Our studies with TRI indicate that blood concentrations are highly correlated with brain concentrations, and thus would also be suitable for such a purpose. This is important since it suggests that blood levels in humans may be used to predict brain levels, and thus the degree of behavioral impairment. There appear to be important limitations, however, to the use of blood and brain concentrations as dose metrics. For example, it can be argued that the differential distribution of solvents in the brain makes it inappropriate to consider the CNS as a single homogenous compartment for kinetic purposes or as a dose metric. Moreover, that behavioral changes in SCOB and locomotor activity may be mediated by a solvent effect other than that exerted on the CNS cannot be discounted. Systematic quantitative relationships between blood and brain solvent concentrations and the accompanying behavioral changes are also hindered by such confounders as acute neuronal adaptation, as was demonstrated in the study of PCE; biphasic response patterns, as was demonstrated for TRI on locomotor activity; and the apparent role of solvent uptake rate, as was evident in TRI's effect on operant responding.

In addition to specific concerns regarding dose metrics, solvent-induced human psychomotor impairment appears to occur at lower blood concentrations than do behavioral effects in animals. This suggests that human psychomotor tests are more sensitive to solvents than behavioral tests employing animals. It is therefore incumbent upon practitioners of behavioral toxicology to devise tests in animals that measure some of the same behaviors that have proven so sensitive in humans. As was demonstrated by comparing dose-response curves for TRI's effect on the operant behavior of singly and repeatedly exposed rats, it is also important to have a thorough knowledge of factors that influence behavior in order to correctly interpret behavioral dose-response data and its suitability for extrapolation to humans.

There is a clear need for an alternative to the current practice of setting exposure limits by applying safety factors to animal data to account for uncertainties inherent in extrapolation on the basis of exposure concentration. The blood and brain concentration time-course data in this dissertation are currently of value for the validation and refinement of PBPK models for animals. The behavioral data sets in this dissertation are important additions to the toxicology literature in that they include the first studies of PCE's effect on operant responding and TRI's effect on the operant responding rats, and a vast expansion of the concentration range used in the one existing study of TRI's effect on locomotor activity. However, the behavioral and pharmacokinetic data will be particularly valuable when used together to validate PBPK models that can predict the tissue pharmacokinetic patterns of halocarbons in experimental animals, and thus the magnitude to neurobehavioral toxicity expected. Validation of a PBPK model using this data could be very useful once the model is scaled up to humans, allowing insight into what exposure levels may or may not produce behavioral effects in humans. To simply apply biochemical and pathological measures of neurotoxicity to PBPK models, in lieu of behavioral measures, would be shortsighted. After all, it can be argued that neurological effects with no functional or behavioral consequences are of little concern, while significant behavioral deficits without currently recognizable neurological bases are still significant deficits. It is certain that in the coming years, there will be exciting opportunities to use pharmacokinetic data to impart a more scientific basis to the assessment of human risk for neurobehavioral toxicity.

XXVI. INTERSPECIES COMPARISONS OF THE REGIONAL BRAIN DISTRIBUTION OF INHALED TRI

It is becoming a common recognition that chemical dose or dose surrogates in tissue, instead of the administered dose, should be employed as effective dosimeter of toxicological responses. This concept encounters a problem, however, when applied to organic solvent neurotoxicity. The brain as the target organ has been shown to display differential capacities of uptake and distributing organic solvents among its various anatomically distinctive regions. While this phenomena has mostly been demonstrated with toluene and ethanol, it would also be true for halogenated hydrocarbons like TRI and TCE, as they too possess similar physicochemical properties. Thus uneven distribution of chemical in brain tissue results in multiplicity of chemical concentration measurements, depending on the method of sampling, and invokes ambiguity in assessing the internal dose-response relationship. While there have been reports on the brain regional based effects of ethanol exposure, there has been limited efforts so far investigating the brain regional disposition of halogenated hydrocarbons; and the potential toxicodynamic consequence of this differential brain disposition is essentially unknown.

Previous reports with toluene and ethanol indicate that volatile organic compounds (VOCs) have uneven distribution in various anatomically distinctive brain regions. No similar study has been reported for halogenated hydrocarbons, to which neurotoxicity is the most prominent health effect. It is known that substantial differences exist in TRI pharmacokinetics between species. In the present study, the uptake and distributions of 1,1,1-trichloroethane (TRI) in the brain tissue of mice and rats were examined. The animals were exposed to TRI at either 3500 or 5000 ppm for up to 2 hrs, and serial sacrificing were done at 10, 30, 60 and 120 min during each of exposure session. Seven brain regions from rats and 3 from mice were sampled, and TRI concentrations in the blood and brain tissues were determined by gas chromatography. Significant difference were found in the TRI concentrations between blood and brain tissue as well as among regional brain tissues in each species. Medulla oblongata in both species demonstrated overall the highest TRI concentrations, while cortex (in both species) and hippocampus (only sampled in rats) showed the lowest TRI concentrations. Substantial differences were also observed between the two species, as the mice exhibited higher capacity to accumulate TRI in blood as well as in the brain tissues. The differential disposition of TRI among the brain regions is seen as mostly driven by the lipid content of various regions. Physiological difference in the respiratory systems of the two species and the physicochemical properties of the chemical favoring diffusion towards lipid-rich compartments might also have facilitated the species differences.

This paper is in the process of being submitted to a peer-reviewed journal, the *Journal of Biochemical Toxicology*. The manuscript is included as section M in the Appendix. The reference for the manuscript is as follows:

You, L., Muralidhara, S., and Dallas, C.E. "Regional brain distributions of trichloroethane in mouse and rats following inhalation exposures." To be submitted to the *Journal of Biochemical Toxicology*.

XXVII. INTERSPECIES COMPARISONS OF THE CYCLIC GMP LEVELS FOLLOWING TRI INHALATION

Two major issues figure prominently in the toxicology of halogenated hydrocarbons. One is their common ability in producing neuro-depression effects upon acute, high dose exposure; another is the extensive metabolism for some of the chemicals in this class. Whereas the acute CNS effects are believed to be caused by the parent compounds, their toxicity in the liver, kidney and their carcinogenicity are most closely associated with one or more metabolites. There are therefore likely separate sequences of cellular and toxicological events leading to the different expressions of toxicity. In light of this, it only seems logical to evaluate both the parent compound-mediated effects and metabolite-mediated effects in order to assess effectively the toxicological significance of a chemical's differential disposition in the brain. The potential candidates of the toxicological endpoints to be used evaluating the neurotoxicity of halogenated hydrocarbons thus need to be (a) quantitative and regionally definable, (b) represent cellular and toxicological events on different time scales with different modes of action. Literature review indicates the brain tissue cyclic guanosine monophosphate (cGMP) and glutathione S-transferases (GSTs) have the potential to be quantitatively reflective of halogenated hydrocarbon neurotoxicities of different kinds of a brain regional basis.

Cyclic GMP is a key intracellular signal transducer for receptor-mediated events. Its metabolism is controlled by a dynamic balance of both guanylate cyclase, which generates cyclic GMAP, and cGMP-dependent phosphodiesterase, which degrades it. It has been demonstrated that exposure to TRI would produce a depression of cyclic GMP level in brain tissue in mice brain areas such as brain stem, cerebral cortex and vermis anterior. With the importance of cGMP as a second messenger in cellular functions, the disturbance of its dynamics is regarded as an expression of toxicity. This effect of TRI is similar to the decrease of cyclic GMP brain level by some neuroactive compound diazepams, which are believed to exert their anxiolytic effect through interacting with GABA receptors. Meanwhile, interactions between volatile organic compounds and GABA receptors has also been observed. In fact, it has been noticed that vast similarity exist between the neuropharmacological effect of organic solvents and classic CNS depressant drugs like the diazepams. One hypothesis outlined a possibility of membrane receptor-mediated CNS effects for organic solvents. The evident neurodepressive effects of halogenated hydrocarbons would be a consequence of their potential role in antagonizing the effects of excitatory amino acid (EAA), such as glutamate and N-methyl-D-aspartate (NMDA), or in activating GABAergic pathways. Whichever case it may be, cyclic GMP would be an effector to reflect the changes in the upstream events.

As it is known that volatile organic compounds (VOCs) exhibit differential dispositions among anatomically discrete brain regions in rodents as well as in humans, potential toxicological consequences of this pharmacokinetic feature were evaluated using measurements of cyclic GMP. With the knowledge of 1,1,1-trichloroethane (TRI) uptake and distribution in the brain regions, cyclic GMP was evaluated due to its known susceptibility to the effects of organic solvents, its pivotal physiological role in perpetuating changes in neurochemical pathways and its possible involvement with neurobehavioral functions, whose disruption is one of the primary health effects associated with solvent exposures. Male CD-1 mice and Sprague-Dawley rats inhaled 5000 ppm TRI for 40 and 100 min in dynamic inhalation exposure chambers, and the brain procured from the animals immediately following terminating by microwave irradiation. Significant decreases in cyclic GMP levels were found

in the cerebellum of both species, 55% and 58%, respectively, relative to the controls. There was a further decrease in both species after 100 min of TRI inhalation. Decreases of cyclic GMP with smaller magnitudes than in cerebellum were seen in the cortex of both species at both time points of measurement. A decrease of cyclic GMP was observed in the medulla oblongata of mice but not in rats after 40 min of exposure. Due to its signal transduction functions, it might be expected that the effects on cyclic GMP could directly impact neurological function. Comparison of the results of this study with the regional brain distribution of TRI and its effects on behavioral performance in previous studies by this laboratory appeared to indicate that alterations in brain cyclic GMP levels may be only involved with the neurobehavioral toxicity of TRI in an indirect fashion, and it appears to not be directly related to regionally differential dispositions of TRI in rodent brain.

The paper is in the process of being submitted to a peer-reviewed journal. The manuscript is included as section N in the Appendix. The reference for the manuscript is as follows:

You, L., Muralidhara, S., and Dallas, C.E. "Effects of inhaled 1,1,1-trichloroethane on the regional brain cyclic GMP levels in mice and rats," to be submitted.

XXVIII. RELATIONSHIP OF THE REGIONAL BRAIN DISTRIBUTION OF INHALED TCE WITH GST CONCENTRATION

Contrast to change in cyclic GMP level which usually represent acute shifts in cellular functions, enzyme induction, involving protein synthesis, often occurs on a much longer time scale. Extensively metabolized in rodent species as well as in humans, TCE is known to interacted with several important metabolic enzymes. The cytochrome P-450 dependent monooxygenases, mainly the isozyme of P-450IIE1, oxidize it into chloral (an aldehyde species), which is then further metabolized to trichloroacetic acid by aldehyde dehydrogenase and to trichloroethanol by NADPH-dependent reductase. The second phase metabolism of TCE involves mainly conjugation reactions catalyzed by glucuronyl transferase, while a minor conjugation pathway involves glutathione transferase. The potential of glutathione S-transferases (GSTs) as marker molecules reflecting broad spectrum of toxicological insults has been suggested, as the GSTs are a family of enzymes that have very characteristic expressions in various organs, tissues and cell types. Their overlap but differential specificity to various substrates make it possible to distinguish particular types of toxicant exposure. But until now, most studies on GSTs have been focused on liver and kidney where the enzyme activity is relatively high; and there is only limited knowledge about the distributions of the various subclasses and isoforms of GSTs in the brain. It is important to understand the physiological heterogeneity of GSTs among the various brain regions for the sake of understanding the metabolic potential of the brain tissue, and more than that, with their structural and functional differences and multiple regulatory mechanisms, changes in GSTs as result of toxicological actions would serve as a very versatile index of toxicodynamic effects of toxicant exposure.

Based on the above understanding, the overall objective of this research project is to evaluate the toxicodynamic relationship between the brain disposition and neurotoxicity of

halogenated hydrocarbons, and it specifically aims at 1) to compare the neurobehavioral response (operant behavioral testing) of mice during TRI exposure with time-matched measurements of the chemical concentrations in blood and brain tissue; and collaterally 2) to examine the possibility that milk intake during animal behavioral training may affect the pharmacokinetics of the testing halogenated hydrocarbon; 3) to delineate the uptake and distributions of TRI in various anatomically distinct brain regions over time during inhalation exposure in both mice and rats and compare the two species; 4) to evaluate the effects of TRI exposure on regional brain tissue cyclic GMP levels; and 5) to study the distribution of various GST isozymes in various brain regions and their relative responsiveness to inhalation exposure of TCE.

This paper is in the process of being submitted to a peer-reviewed journal, *Fundamental and Applied Toxicology*. The manuscript is included as section O in the Appendix. The reference for the manuscript is as follows:

You, L., Muralidhara, S., and Dallas, C.E. "Rat regional brain dosimetry of trichloroethylene and its toxicodynamic relationship with glutathione S-transferase isozymes." To be submitted to *Fundamental and Applied Toxicology*.

The tissue dosimetry of trichloroethylene (TCE) in Sprague-Dawley rat brain regions and extracerebral organs was examined and compared to the effects of TCE inhalation on glutathione S-transferase (GST) activity and its isoform portion levels in the brain. From the physicochemical properties of TCE and available data on the pharmacokinetic properties of similar compounds, it was hypothesized that TCE could exhibit differential regional brain concentrations in this target organ during the frame in which TCE-induced neurotoxicity has been demonstrated. Male Sprague-Dawley rat received either a single 2 hour inhalation exposure to 4500 ppm or three consecutive daily exposure to 2500 ppm for 2 hr each day. TCE uptake in brain was rapid following inhalation exposure, reaching levels more than twice that in the blood in the same time period. Regional brain differences in TCE concentration were manifested, with medulla oblongata and striatum with relatively higher levels and cortex and cerebellum with lower concentrations. The effects of TCE inhalation on GSTs were not distinguishable between the repeated 2500 ppm exposure and the single 4500 ppm exposure groups. While most changes in the GST subunit levels were increases in the later (14 days) time point of sampling, decreases of the protein levels were also observed. The relatively small magnitudes of these changes, in addition to the fact that there lacked an apparent correlation between the rank order of total TCE doses in the brain regions and the effects they brought about to the GSTs, indicates the possibility that the effects of TCE exposure on GSTs are nonsystematic and limited.

XXIX. SUMMARY AND CONCLUSIONS, DISSERTATION OF LI YOU, PH.D. (1996)

Exposure to short chain aliphatic, halogenated hydrocarbons, a group of widely used organic solvents, pose serious threat to human health. In the efforts to increase the reliability of health risk assessments specific to various exposure conditions, pharmacokinetic knowledge is seen as essential in reducing the acknowledged uncertainties associated with

this process. An internal dose approach is advocated to facilitate the extrapolation of toxicological data between various exposure scenarios.

This project was designed to evaluate the toxicodynamic relationship between the brain disposition and neurotoxicity of halogenated hydrocarbons. Five specific studies were conducted to fulfill the mission: 1) comparative study on operant response of mice during 1,1,1-trichloroethane (TCE) exposure with time-matched measurements of TCE concentrations in blood and brain tissue; and collaterally 2) examining the possibility that milk intake during animal behavioral training may affect the pharmacokinetics of the testing chemical; 3) delineating the dosimetry of TCE in various anatomically distinct brain regions over time during inhalation exposure and comparing differences between mice and rats; 4) examining the effects of TCE exposure on cyclic GMP levels in regional brain tissues; and 5) studying the distribution of various glutathione S-transferase (GST) isozymes in various brain regions and their relative responsiveness to inhalation exposure of TCE.

Extensive TCE pharmacokinetic data has been obtained through this project. Major features included dissimilar blood and organ tissue concentrations, high accumulation of the compounds in lipid rich organs, significant differences in chemical concentrations among brain regions and between species. Marked toxicological effects of halogenated hydrocarbons have been demonstrated, as evidenced by depression of operant response, depression of cyclic GMP level in brain tissue, and regional specific effects on GST isozymes regulations.

This project is an attempt to bridge pharmacokinetics with both behavioral and biochemical aspects of neurotoxicity induced by halogenated hydrocarbons. This is the first extensive effort known to study the regional brain disposition of halogenated hydrocarbon and its toxicodynamic consequence. The data presented herein will be helpful in pharmacokinetic and pharmacodynamic modeling of the health effects for these chemicals.

This research project consists of two major components: chemical disposition and neurotoxicity of halogenated hydrocarbons. The first focus aimed at investigating the possibility that halogenated hydrocarbons might, like some other volatile organic solvents, have differential uptake and distribution among anatomically distinct brain regions. The second focus of the research centered on the manifestations, or the toxicodynamic consequences of halogenated hydrocarbon disposition. It was expected that, through a careful evaluation of neurotoxicity in relation to internal dose of halogenated hydrocarbons at the tissue level, this research endeavor would provide critical data and toxicological insights helpful in reducing the uncertainties associated with conventional approaches of health risk assessment concerning various halogenated hydrocarbon exposures.

A multifaceted approach, including neurobehavioral testing and neurochemical and enzymatic analysis, was adapted to investigate the neurotoxicity of halogenated hydrocarbons. Operant behavioral technique was utilized to assess behavioral response of mice to the exposure of 1,1,1-trichloroethane; assay of cyclic GMP brain tissue level was used to assess the potential of TCE altering neurochemistry on a regional brain basis; and glutathione S-transferase isoforms was measured to evaluate the impact of halogenated hydrocarbons on enzyme induction. In regard to tissue chemical disposition, both TCE and TCE were analyzed in brain regions and extracerebral organs. A comparison was made between mice and rats in their regional brain uptakes and distributions of TCE. In conjunction

to the operant testing, a study was carried out to examine the pharmacokinetic validity for the use of milk as behavioral reinforcer in relation to the testing of halogenated hydrocarbon behavioral toxicity.

The operant study demonstrated a neurobehavioral depression effect of TRI. The fact that the effect was seen at 5000 ppm but not 3500 ppm inhalation exposure suggests several factors other than the exposure dose and the measured brain tissue concentrations may contribute to the acute CNS effects of TRI. Those factors included the particular combination of animal species, the length of exposure, and the schedule of operant reinforcement, as well as the difference in the rate of TRI uptake at the two exposure levels. The use of milk as behavioral reinforcer in the animal training and testing protocol was found not to change TRI tissue concentrations, thus removing the concern that milk intake in that order could potentially change the body fat composition of the animal and alter the pharmacokinetics of the testing compounds. A high level of TRI accumulation in the fat tissue was a demonstration of the high lipophilicity of halogenated hydrocarbons, which was about 20 times higher in the fat than any other body tissue measured in the 100 min inhalation study.

Uptake of both TRI and TCE were different among various brain regions. In general, the medulla oblongata, midbrain and striatum were at the high end of a spectrum of concentration, while cortex, cerebellum and hippocampus were at the low end. But the exact rank order of chemical concentration in the brain regions changes with dose and duration of exposure. All the tissue concentrations were higher than in the blood, and the eliminations from the blood as well as from tissues were fast following the exposure. With respect to brain tissue level cyclic GMP, there were large physiological differences among brain regions and between rats and mice. In both species, TRI exposure changed the cyclic GMP levels, and the largest were reductions (> 100%) in cerebellum, which had the highest control values. Marked heterogeneity in the brain was also demonstrated with glutathione S-transferases (GSTs). Total GST activity levels were shown to be different in the brain regions. The difference in GST isozyme distributions in the brain regions were also evident. The effects of TCE, with different combination of exposure schemes, were mostly seen in midbrain and striatum areas of the brain and in the liver. The effects of enzyme induction in some cases while reduction in others indicate a possibility of multiple active species of the compound and/or multiple regulatory mechanisms for GST levels being affected in various tissues. No single GST subunit had a consistent responsiveness to TCE exposure in all the tissues measured. The mixed reactions of the GSTs to TCE suggest that the potential of GSTs as biomarkers or dose surrogates for halogenated hydrocarbon exposure may be limited.

There is a lack of apparent correlation between the halogenated hydrocarbon concentrations in the tissues and the effects of TRI and TCE on cyclic GMP and GST isozymes in corresponding brain regions. This suggests a possibility the effects seen may not be directly propagated by the primary effectors of the chemical, or the responsible entities may not be the parent compounds, which were the only ones measured in the studies. A better understanding for the mechanism of action for the halogenated hydrocarbon induced acute neurotoxicity will greatly help to delineate these relationships. It seems plausible that the CNS physiological heterogeneity may have a role shaping various manifestations of chemical toxicology. At the level of pharmacokinetics, for instance, the non-uniform lipid contents in various brain regions appear largely responsible to the differential dosimetry of halogenated hydrocarbons. But until we know more about the chemical effects on cellular and molecular

events, it will be difficult to assess the full significance of this differential dosimetry of toxicant in the brain.

A major task to health risk assessment continues to be the establishing of a dose-response relationship for chemical exposure and then reliably applying the knowledge of the relationship to various conditions. Physiologically based pharmacokinetic (PBPK) approaches have lead the way in realistically extrapolating of chemical concentrations between dose levels, species, and exposure route and in accurately predicitng toxicant dose levels associated with a particular exposure. These achievements have largely based on the progresses in the understanding of the factors determining chemical absorption, distribution, metabolism and elimination. Physiologically based pharmacodynamic (PBPD) modeling has similar potentials in predicitng specific toxic reactions. The development and utility of PBPD methodology will largely dependent on the state of the understanding for the mechanisms of toxicant actions, which are still far from adequate in many cases, such as for halogenated hydrocarbons. This dissertation research project is a needed addition to the efforts in exploring concepts, testing hypotheses and accumulating data -- activities that will lead to continuing progress in our understanding of health risk associated with chemical exposures.

XXX. COLLABORATIVE ARRANGEMENTS

The proposed project has been conducted at the Department of Pharmacology and Toxicology (P & Tx) and the Department of Pharmaceutics in the University of Georgia (UGA) College of Pharmacy. The Principal Investigator has been Dr. Cham E. Dallas (CED), who has been responsible for overall coordination of the project. He has provided a 20% commitment to this project. In addition to coordinating the project, CED personally conducted all of the respiratory elimination studies that were done. In that effort he developed the exposure system for the direct kinetic determinations of halocarbons, along with monitoring of respiratory parameters. Under CED's direction, the assay was developed for measuring halocarbon levels in the tissues of animals following exposure, which has been of significant utility in the present investigation. A primary focus of CED's studies has been to provide data sets for the development and validation of physiologically-based pharmacokinetic (PBPK) models. Dr. James V. Bruckner (JVB) has provided a 10% commitment to the project as a Co-Principal Investigator. He has directed a number of research projects on the oral toxicity and pharmacokinetics of volatile organic compounds over the past 10 years. JVB is also experienced in the application of pharmacokinetic data to risk assessments, having served on a number of committees and advisory groups for federal agencies concerned with health effects of VOCs. Dr. James M. Gallo (JMG) has served as a Co-Investigator (5% commitment), and provided expertise in the field of pharmacokinetics (PK). The major focus of his work has been the physiologically-based pharmacokinetic (PBPK) modeling, including the derivation of methods for estimation of mass transfer coefficients and partition coefficients for PBPK models. JMG had primary responsibility for design of PK studies and analysis of data, development and refinement of PBPK models, and assessment of the model's ability to predict halocarbon disposition in humans. Dr. Randall Tackett (RT) served as a Co-Investigator on the project (10% commitment), and was responsible for the kinetic experiments in the dog in this project. RT heads an active laboratory staffed by postdoctoral associates and graduate students, in which a number of toxicodynamic studies in the dog and the rat have been conducted. Dr. Tom Reigle (TR) also served as a Co-Investigator on the

project (20% commitment). TR provided valuable assistance in the selection and purchase of the appropriate testing equipment that can be used for both rats and dogs, and was involved in the design and conduct of all the neurobehavioral studies.

Two doctoral students completed their Ph.D. degrees with research supported by this grant. Mr. Alan Warren (AW) is a doctoral student who completed his dissertation research and graduated in the Summer of 1995. Alan is the recipient of a three-year award from the Department of Defense, managed by the Southeastern Center for Electrical Engineering Education (SCEEE). This award provides for his graduate assistantship stipend and approximately \$2000 annually for travel and minor expenses. As the SCEEE award coincides almost exactly with the period of this Air Force grant, this is an important (and much appreciated) collaborative effort. AW was in charge of the conduct of all of the neurobehavioral studies conducted during the first year of the grant. He has personally been involved in the development of the neurobehavioral testing protocols, and has provided a very perseverant effort toward the success of this critical part of the project. He has been assisted by Warren Christmus, a Pharmacy student who has worked approximately half-time over two years on the neurobehavioral training. Mr. Li You, also completed his dissertation on this project, involving neurochemical and neurobehavioral correlations with regional brain differences in several rodent species. He graduated with his Ph.D. in April, 1996. Technical and clerical assistance was also provided by Wade Meredith, a masters student in the Department of Pharmacology and Toxicology.

Dr. Xiao Mei Chen (XMC) has served as a full-time postdoctoral associate on the project. XMC has a medical degree from the People's Republic of China, and has worked in the current Air Force project since its inception. She was successful in her work in the development of the assay for the measurement of halocarbons in the tissues of exposed animal and has conducted these tissue measurements thus far for ia and po exposures for PER and TET in rats and dogs. Dr. Peter Varkonyi (PV) is a postdoctoral associate from Hungary who has worked "hands-on" with the development of the PBPK models for halocarbon pharmacokinetics. Mr. Srinvasa Muralidhara has been employed part-time (25%) on the project. He participated in the analysis of halocarbons in biological samples, computer programming, the conduct of inhalation studies of dynamic exposure chambers, and the compiling of laboratory records.

XXXI. INTERACTION WITH DOD LABORATORIES

There is currently much debate among behavioral toxicologists as to the interpretation of neurobehavioral data and the appropriateness of specific methods used to collect it. In an effort to participate in this scientific debate and stay abreast of developments in the field of neurobehavioral toxicology, Dr. Cham Dallas and Mr. Alan Warren attended a conference entitled, "Toxicological Interpretation of Neurobehavioral Data." The conference was held at the University of Rochester Medical Center, Rochester, NY., on July 22-24, 1992. Attendance at this conference provided the opportunity to solicit the opinions of prominent behavioral toxicologists from academia, government, and the private sector. The program from the Rochester conference is included as E-25 through E-30. Additionally, Mr. Warren recently visited the toxicology division at Wright-Patterson Air Force Base (WPAFB), an opportunity afforded him as a recipient of a Department of Defense Science and Engineering

Fellowship. While at WPAFB, Mr. Warren met with his Air Force Laboratory mentor, Dr. Jeff Fisher, as well as other Air Force and contract scientists with expertise in pharmacokinetic modeling and toxicodynamics. Other opportunities to discuss neurobehavioral toxicology should arise at the next meeting of the Southeastern Society of Toxicology scheduled for October 15-16 in Athens, Ga. The meeting will include a symposium entitled "Neurotoxicology: Experimental, Clinical and Environmental Aspects." Mr. Warren also arranged for Dr. Christopher Newland of Auburn University to deliver a seminar to the Department of Pharmacology and Toxicology, University of Georgia, on his use of schedule-controlled operant behavior in toxicological studies. After completing his Ph.D., Mr. Warren went on to accept a postdoctoral position with Dr. Fisher at WOAFB, thus continuing this useful and productive interaction with DOD.

APPENDIX A

Dallas, C.E., Gallo, J.M., Ramanathan, R., Muralidhara, S., and Bruckner, J.V. "Physiological pharmacokinetic modeling of inhaled trichloroethylene in rats." *Toxicology and Applied Pharmacology* 110: 303-314 (1992).

Physiological Pharmacokinetic Modeling of Inhaled Trichloroethylene in Rats^{1,2}

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Physiological Pharmacokinetic Modeling of Inhaled Trichloroethylene in Rats. DALLAS, C. E., GALLO, J. M., RAMANATHAN, R., MURALIDHARA, S., AND BRUCKNER, J. V. (1991). *Toxicol. Appl. Pharmacol.* **110**, 303-314. The pharmacokinetics of trichloroethylene (TCE) was characterized during and following inhalation exposures of male Sprague-Dawley rats. The blood and exhaled breath TCE time-course data were used to formulate and assess the accuracy of predictions of a physiologically based pharmacokinetic (PB-PK) model for TCE inhalation. Fifty or 500 ppm of TCE was inhaled by unanesthetized rats of 325-375 g for 2 hr through a miniaturized one-way breathing valve. Repetitive samples of the inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently during and for 3 hr following the exposures and analyzed for TCE by headspace gas chromatography. Respiratory rates and volumes were continuously monitored and used in conjunction with the pharmacokinetic data to delineate uptake and elimination profiles. Levels of TCE in the exhaled breath attained near steady-state soon after the beginning of exposures, and were then directly proportional to the inhaled concentration. Exhaled breath levels of TCE in rats were similar in magnitude to values previously published for TCE inhalation exposures of humans. Levels of TCE in the blood of the 50 ppm-exposed animals also rapidly approached near steady-state, but blood levels in the 500 ppm-exposed animals rose progressively, reaching concentrations 25- to 30-fold higher than in the 50 ppm group during the second hour of exposure. The 10-fold increase in inhaled concentration resulted in an 8.7-fold increase in cumulative uptake, or total absorbed dose. These findings of nonlinearity indicate that metabolic saturation ensued during the 500 ppm exposure. The PB-PK model was characterized as blood flow-limited with TCE eliminated unchanged in the exhaled breath and by saturable liver metabolism. The uptake and elimination profiles were accurately simulated by the PB-PK model for both the 50 and 500 ppm TCE exposure levels. Such a model may be quite useful in risk assessments in predicting internal (i.e., systemically absorbed) doses of TCE and other volatile organics under a variety of exposure scenarios. © 1991 Academic Press, Inc.

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Trichloroethylene (1,1,2-trichloroethylene, TCE) is a volatile organic compound (VOC) which has been widely used as a surgical anesthetic, fumigant, extractant in food processing, metal degreaser, dry cleaning agent, and solvent in other commercial applications. It has been estimated that of 3.5 million persons believed to be occupationally exposed to TCE in the U.S., at least 100,000 workers are exposed full-time, and that two-thirds of these

are in work environments where there are not adequate control measures (NIOSH, 1978). Although excessive exposures to TCE vapors have resulted in cardiac arrhythmias and in central nervous system depression, most occupational exposures do not result in apparent manifestations of toxicity (Defalque, 1965; NIOSH, 1973; U.S. EPA, 1985). There is considerable concern, however, that TCE may be a human carcinogen. TCE has been reported to produce an increased incidence of hepatocellular carcinoma in B6C3F1 mice subjected daily for their lifetime to high oral doses of the chemical (NCI, 1976; NTP, 1983). More recent studies have also shown that TCE can be carcinogenic in animals upon inhalation exposure (Fukuda *et al.*, 1983; Maltoni *et al.*, 1988).

Assessment of toxic and carcinogenic risks of exposure to TCE and other VOCs has become a subject of major importance over the last decade. Although it has formerly been common practice to calculate risk estimates on the basis of administered dose-toxicity/tumor incidence data, it is now recognized that the internal, or target organ dose is a more accurate and direct determinant of the magnitude of injury. The dose of chemical actually reaching a target organ is dependent upon kinetic processes which may vary considerably with the administered dose, route of exposure, and animal species. Thus, recognition and use of pharmacokinetic data can substantially reduce uncertainties inherent in the route-to-route, high-dose to low-dose and species-to-species extrapolations often necessary in risk assessment (Gehring *et al.*, 1976; Clewell and Andersen, 1985; NRC, 1987).

There have been a relatively large number of studies of the pharmacokinetics of TCE in humans, but data on the time-course of alveolar and blood levels during ongoing inhalation exposures are quite limited. Most human studies have focused on the elimination of TCE and its major metabolites postexposure (Stewart *et al.*, 1970; Kimmerle and Eben, 1973a; Monster *et al.*, 1976; Sato *et al.*, 1977). Additional studies to obtain TCE time-course

profiles are increasingly limited by the ethical question of exposing persons to a potential human carcinogen. Thus, investigations utilizing laboratory animals must be largely relied on to provide such information.

Surprisingly, there are relatively few data available in the literature on the time-course of TCE or its metabolites in laboratory animals inhaling the chemical. Most existing studies are limited to the elimination phase following exposure (Kimmerle and Eben, 1973b; Nakajima *et al.*, 1988; Fisher *et al.*, 1989). Technical difficulties with measuring solvent uptake and respiratory functions serially in small animals during inhalation exposures have hindered accurate definition of TCE uptake and elimination profiles. Prout *et al.* (1985) did investigate the time-course of TCE and its major metabolites in the bloodstream of mice and rats given a 1,000 mg/kg oral dose of TCE in corn oil. The study results are useful qualitatively in that they reveal that TCE undergoes much more extensive first-pass metabolism in the mouse than in the rat. The results are of limited use quantitatively, however, in that blood was collected from only one animal at each time-point. Balance studies in mice and rats administered [¹⁴C]TCE orally (Prout *et al.*, 1985; Dekant *et al.*, 1986) and by inhalation (Stott *et al.*, 1982) confirm that mice have a higher TCE metabolic capacity than do rats. In each study, ¹⁴C levels in animal tissues were measured only at a single time (i.e., 50 or 72 hr) postexposure. Thus, blood and tissue TCE concentration versus time data that are presently available are not adequate to delineate the internal dose of TCE received during inhalation exposures.

Physiologically based pharmacokinetic (PB-PK) models have been formulated for a number of VOCs, in an effort to better understand and forecast the dynamics of the chemicals in the blood and tissues of laboratory animals and humans. The NRC (1986) was the first to describe the use of a PB-PK model for TCE in route-to-route and rat-to-human extrapolations. Bogen (1988) applied the styrene PB-PK model of Ramsey and Andersen (1984) to

predict relationships between the administered dose of TCE, the toxicologically effective dose, and the risk of cancer in humans. Experimental data were not supplied in either case, however, to test the fidelity of the TCE model predictions. Fisher *et al.* (1989) recently developed a PB-PK model to describe the dynamics of TCE and trichloroacetic acid in pregnant rats exposed to TCE by inhalation and ingestion. The model simulations compared favorably with the limited blood TCE concentration time data which were available to the investigators.

In consideration of the foregoing, the objectives of the present investigation were to: (a) quantify the rate and magnitude of TCE uptake and elimination over time during the course of TCE inhalation exposures of rats, (b) accurately define blood and exhaled breath TCE concentration versus time profiles during and after the exposures, and (c) formulate a PB-PK model for inhalation of TCE based on the observed time-course data.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. There were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formula Chow were provided *ad libitum*. The rats were used after at least a 14-day acclimation period, at which time their body weight ranged from 325–375 g. TCE exposures were initiated at approximately the same time each day (1000 to 1200 hr).

Test material. Trichloroethylene (TCE), of >99.99% purity, was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). The purity of the solvent was verified by gas chromatography.

Animal preparation. All rats were surgically implanted with an indwelling carotid artery cannula, which exited the animal at the back of the neck. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml), acepromazine maleate (10 mg/ml), xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v:v:v). The cannulated animals were maintained in a harness and pulley system that allowed relative freedom of movement in metabolism cages during a 24-hr recovery period after surgery.

Inhalation exposures. A known concentration of TCE was generated within a 70-liter gas sampling bag (Calibrated Instruments, Ardsley, NY) by injecting the appropriate quantity of TCE into the bag filled with air. Uniform dispersion of the vapor was insured by a magnetic stirring bar within the bag. The bag was then connected in series by Teflon tubing with a pneumotachograph, a miniaturized one-way breathing valve (Hans Rudolph, Inc., St. Louis, MO), and an empty 70-liter gas collection bag. The latter bag served as a reservoir to collect exhaled gas. The breathing valve was attached to a face mask designed to fit the rat, so that the valve entry port was directly adjacent to the nares of the test animal. Thus, separate and distinct airways for the inhaled and exhaled breath streams were established. Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. Each cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneva, Switzerland). The face mask was held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. This basic inhalation exposure setup has been illustrated previously by Dallas *et al.* (1989). TCE exposures were initiated only after stable breathing patterns were established during a 1-hr acclimation period. During the 2 hr of TCE exposure and for 3 hr afterward, serial inhaled and exhaled breath samples were taken at approximately the same time as blood samples from the carotid artery cannula. The blood samples were then analyzed for TCE content by headspace gas chromatography, whereas the air samples were injected directly into the gas chromatograph.

Respiratory measurements and calculations. The respiration of each animal was continuously monitored according to previously published methods (Dallas *et al.*, 1983, 1986). The airflow created by the animal's inspiration was recorded both during and following TCE inhalation exposure in terms of minute volume (volume of respiration per minute, or V_E), respiratory rate (f), and tidal volume (V_T). Mean values for these parameters were obtained by averaging the measurements taken at 10-min intervals in individual animals during the 2-hr exposures. Mean \pm SE values for the 500 ppm-exposure group ($n = 6$) were: $V_E = 218.0 \pm 20.2$ ml/min, $f = 128.4 \pm 7.1$ breaths/min, and $V_T = 1.71 \pm 0.15$ ml. Means \pm SE for the 50 ppm group ($n = 6$) were: $V_E = 268.9 \pm 15.5$ ml/min, $f = 132.0 \pm 7.3$ breaths/min, and $V_T = 2.12 \pm 0.20$ ml.

Calculations of TCE uptake and elimination were conducted utilizing the equations presented in a previous VOC inhalation study in rats (Dallas *et al.*, 1989). Since the V_E and the exhaled breath TCE concentration at each sampling point were measured, subtraction of the quantity of TCE exhaled by the animal from the amount inhaled yielded an estimation of the quantity of TCE taken up during sequential sampling periods (cumulative uptake). The percentage uptake during each exposure period was determined by dividing the cumulative uptake by the total inhaled dose for that time period. The statistical signifi-

cance of differences between the 50 and 500 ppm animals in percentage uptake at each time point was assessed by an unpaired *t* test, with $p < 0.05$ chosen at the minimum level of significance.

PB-PK model. A PB-PK model was used to describe the disposition of TCE in the rat (Fig. 1). It was assumed that a blood-flow-limited model was adequate to characterize the tissue distribution of TCE. Previous PB-PK models for VOCs have utilized blood-flow-limited organ representations (Ramsey and Andersen, 1984; Angelo and Pritchard, 1984; Dallas *et al.*, 1989). Compartmental volumes and organ blood flows were obtained from the values used by Ramsey and Andersen (1984) for rats, and scaled to 340 g, the mean body weight of rats utilized in the present study. Tissue:blood partition coefficients that characterize the extent of tissue TCE uptake were obtained from Andersen *et al.* (1987). The Michelis-Menton parameters, V_{\max} and K_m , describing the rate of TCE metabolism, were initially estimated from Andersen *et al.* (1987), and were $K_m = 0.25 \mu\text{g}/\text{ml}$ and $V_{\max} = 183.3 \mu\text{g}/\text{kg}/\text{min}$. When scaled to the 340-g rat used in the current study, $V_{\max} = 82.0 \mu\text{g}/\text{min}$. The final value of V_{\max} , set equal to $75.0 \mu\text{g}/\text{min}$, provided good agreement between observed and predicted blood TCE concentrations. Dif-

ferential mass balance equations, incorporating the parameters listed in Table 1, that described the transport of TCE in the rat as depicted in Fig. 1, were numerically solved with the ACSL (Advanced Continuous Simulation Language) computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted blood and exhaled breath TCE concentrations as a function of time.

Analysis of TCE in air and blood. The concentration of TCE in the inhaled and exhaled air was measured with a Tracor MT560 gas chromatograph (Tracor Instruments, Austin, TX). Standards were prepared in each of four 9-liter bottles equipped with Teflon stoppers with needles from which air samples could be taken by syringe. Analyses for the 500 ppm exposures were conducted using a flame ionization detector (FID), while the analyses for the 50 ppm inhalation exposures were conducted using an electron capture detector (ECD). The detection limits for TCE in air by FID and ECD were 0.5 and $0.003 \mu\text{g}/\text{ml}$, respectively. The ECD detector was employed for the 50 ppm exposures, since most of the postexposure exhaled breath concentrations in these animals were below the FID detection limit. In either case, air samples were procured with a gas-tight, 1-ml syringe and injected directly onto

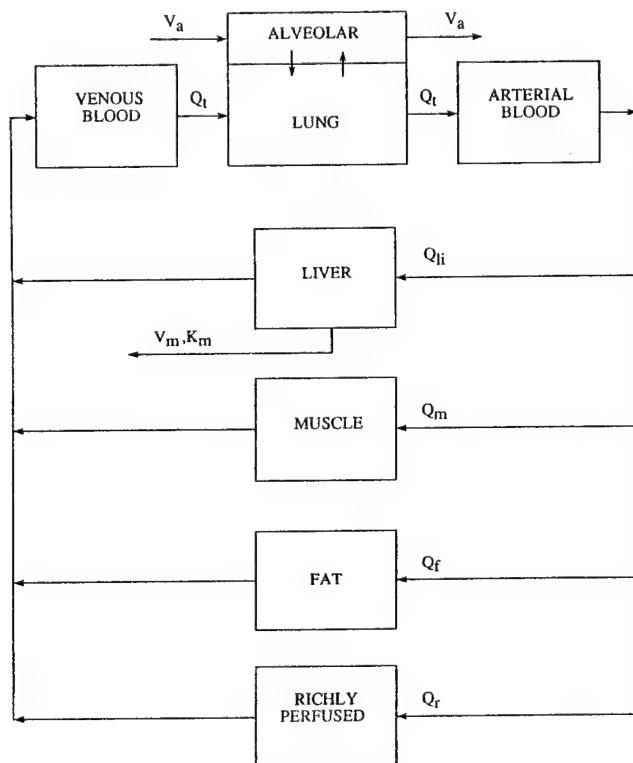


FIG. 1. Diagram of the physiological pharmacokinetic model used to simulate the uptake and elimination of inhaled TCE. The symbols and parameters used to describe the model are included in Table 1.

TABLE 1

PARAMETERS FOR THE PHYSIOLOGICAL PHARMACOKINETIC MODEL OF INHALED TCE IN THE RAT (340 g)

Parameter	Value
Alveolar ventilation rate (ml/min), VR_a	134.5 (50 ppm exposure) 109 (500 ppm exposure)
Inhaled gas concentration ($\mu\text{g}/\text{ml}$)	0.272 (50 ppm exposure) 2.69 (500 ppm exposure)
Alveolar mass transfer coefficient	500 ml/min
Blood flows (ml/min)	
Cardiac output, Q_b	106.4
Fat, Q_f	9.4
Liver, Q_{li}	39.8
Muscle, Q_m	12.8
Richly Perfused, Q_r	44.4
Tissue volumes (ml)	
Alveolar	2.0
Blood	25.4
Fat	30.5
Liver	13.6
Lung	3.97
Muscle	248.0
Richly Perfused	15.2
Partition coefficients	
Blood:Air	21.9
Fat:Blood	25.3
Liver:Blood	1.24
Muscle:Blood	0.46
Richly Perfused:Blood	1.24
Metabolism Constants	
V_{max} ($\mu\text{g}/\text{min}$)	75.0
K_m ($\mu\text{g}/\text{ml}$)	0.25

an 8-ft \times $\frac{1}{8}$ -in. stainless-steel column packed with 0.1% AT 1000 on GraphPak. Operating temperatures were 150°C, injection port; 200°C, FID detector; 350°C, ECD detector; and 110°C, isothermal column operation. When using the ECD, gas flow rates of 40 ml/min were employed for nitrogen (carrier gas), with an additional make-up gas flow rate to the detector of 30 ml/min.

TCE levels in the blood were measured by gas chromatographic headspace analysis. Blood samples were withdrawn from the arterial cannula via a stopcock into a 1-ml syringe. Depending on the anticipated blood TCE concentration, from 25 to 200 μl of the blood was taken from the stopcock with an Eppendorf pipet and transferred to chilled headspace vials (Perkin-Elmer, Norwalk, CT). These vials were capped immediately with PTFE-lined butyl rubber septa and washers and tightly crimped. Each sample vial was then placed into the HS-6 auto-sampler unit of a SIGMA 300 gas chromatograph (Perkin-Elmer,

Norwalk, CT), where it was heated to a preset temperature by a high precision thermostat device. A predetermined volume of the vapor was then injected automatically into the column for analysis. Standard curves were generated on each day that measurements were conducted by injection of known quantities of TCE into headspace vials for subsequent analysis. The column used was an 8-ft \times $\frac{1}{8}$ -in. stainless-steel column packed with FFAP chromasorb W-AW (80–100 mesh). Operating temperatures were 250°C, injection port; 350°C, ECD detector; and 80°C column oven. The carrier gas was 5% argon–methane, at a flow rate of 40 ml/min, with a make-up gas flow rate to the detector of 20 ml/min.

RESULTS

While 50 and 500 ppm were the target TCE inhalation concentrations, the actual concentrations inhaled by the animals were determined by analysis of air samples taken from the airway immediately adjacent to the breathing valve. Mean ($\pm\text{SE}$) inhaled TCE concentrations for the six rats in each group were 499.8 ± 12.7 ppm for the 500 ppm exposures and 50.7 ± 0.8 ppm for the 50 ppm exposures.

The time-courses of TCE concentrations in the exhaled breath and arterial blood were delineated during and for 3 hr following 2-hr exposures of rats to 50 (Fig. 2) and 500 ppm (Fig. 3) TCE. TCE was readily absorbed from the lungs into the arterial circulation, as reflected by relatively high blood levels at the initial sampling time (i.e., 1 min). The concentration of TCE increased rapidly in the blood of the 50 ppm animals, reaching near steady-state levels within approximately 25 min. In contrast, blood levels in the 500 ppm animals increased steadily, but did not reach steady-state after 2 hr of TCE inhalation. The arterial concentrations were not proportional to the inhaled concentrations. Blood levels during the latter hour of exposure were 25–30 times higher in the 500 than in the 50 ppm group. Exhaled breath levels of TCE increased more rapidly than did blood levels after the initiation of exposures, the former attaining near steady-state within 10–15 min. The exhaled breath TCE concentrations remained

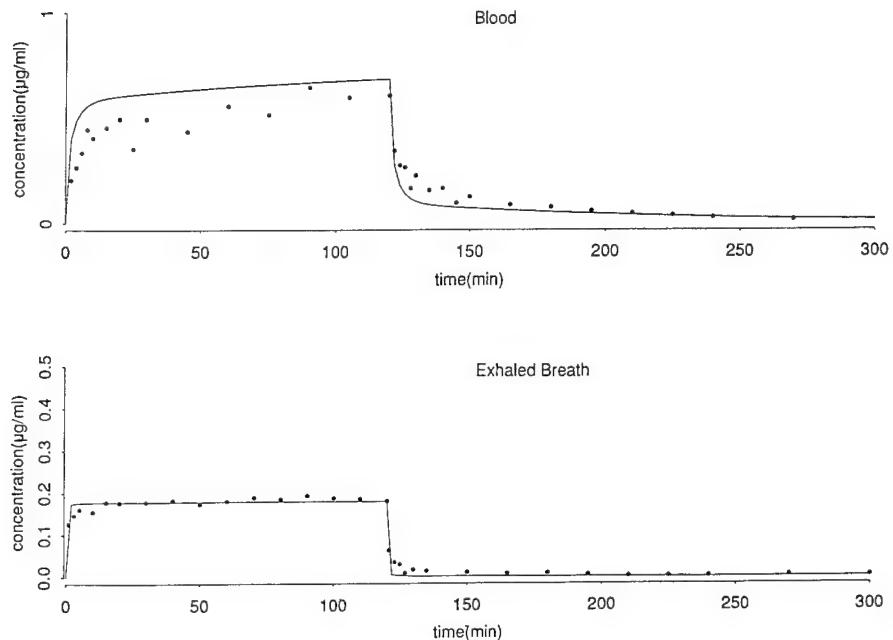


FIG. 2. Observed (●) and model-predicted (—) TCE concentrations in the arterial blood and exhaled breath of rats during and following a 2-hr, 50 ppm inhalation exposure. Each point represents the mean value for six rats.

relatively constant for the 2-hr duration in both exposure groups. Unlike blood levels, the exhaled breath levels at near steady-state were directly proportional to the inhaled concentrations. Mean TCE concentrations in the expired air during the last 1.5 hr of the 2-hr exposures were 34.6 ± 1.1 and 340.8 ± 10.6 ppm ($\bar{x} \pm SE, n = 6$) in the 50 and 500 ppm groups, respectively.

The disappearance of TCE from the blood generally paralleled that in the expired air postexposure, though some disparity was observed. Concentrations of TCE measured in the exhaled breath and blood initially decreased very rapidly after exposures ceased. As can be seen in Figs. 2 and 3, the patterns of elimination differed, in that blood levels diminished more slowly than exhaled breath levels during the first 30 to 45 min postexposure. This difference was most pronounced in the 500 ppm group. Thereafter, TCE was eliminated from the blood and breath at comparable rates. The TCE levels were not mon-

itored long enough postexposure to accurately define the terminal elimination half-lives.

The PB-PK model accurately described the uptake and elimination of TCE in both the blood and expired air. Model-generated exhaled breath and blood TCE concentrations are represented by solid lines in Figs. 2 and 3. The predictions of exhaled breath levels during inhalation were in close agreement with the direct measurements of expired TCE at both exposure levels. Postexposure exhaled breath concentrations were well simulated for the 50 ppm group, and only slightly underpredicted during the first 45 min for the 500 ppm group. The progressive increase in blood concentration during the 2-hr, 500 ppm exposure was accurately forecast by the model. The pattern of uptake of TCE into the blood of the 50 ppm animals was adequately described, although the TCE concentrations were slightly overpredicted (i.e., by about 0.1 µg/ml). The model predicted a more rapid postexposure decline in blood levels than was observed during the

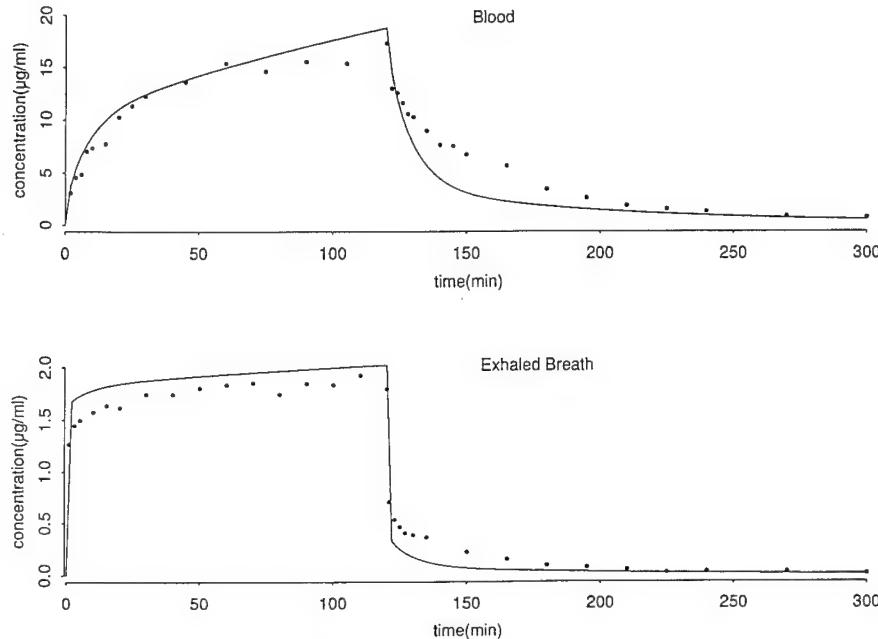


FIG. 3. Observed (●) and model-predicted (—) TCE concentrations in the arterial blood following a 2-hr, 500 ppm inhalation exposure. Each point represents the mean value for six rats.

first hour in the 500 ppm group. Predicted and observed postexposure blood concentrations compared favorably, however, for the 50 ppm rats.

Percentage systemic uptake of TCE was time- but not concentration-dependent (Fig. 4). Uptake exceeded 90% during the first 5 min in both exposure groups, but decreased rapidly over the next 30 min. Thereafter, there was a relatively slow decline in uptake for the remainder of the 2-hr exposure. Percentage uptake appeared to be somewhat higher in the 50 than the 500 ppm animals during much of the first hour, though at no time point was there a statistically significant difference. Percentage systemic uptake values ($\bar{x} \pm \text{SE}$, $n = 6$) during the second hour of exposure for the 50 and 500 ppm groups were $69.9 \pm 0.5\%$ and $71.1 \pm 0.8\%$, respectively. There was a total cumulative uptake ($\bar{x} \pm \text{SE}$) during the 2-hr period of 2.96 ± 0.32 mg, or 8.4 mg/kg , in the 50 ppm animals and 24.3 ± 1.2 mg, or 73.3 mg/kg , in the 500 ppm animals (Fig. 5).

DISCUSSION

There is a lack of definitive information on the systemic uptake and disposition of inhaled TCE during exposures, largely due to technical difficulties in accurately monitoring TCE lev-

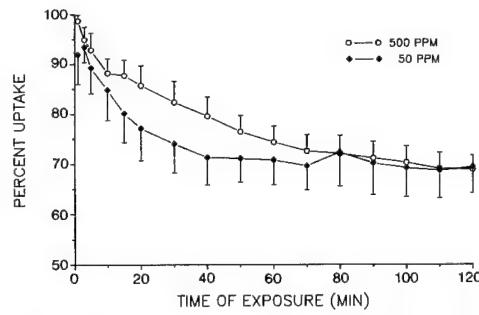


FIG. 4. Percentage systemic uptake of TCE over time during inhalation exposures. Rats inhaled 50 or 500 ppm TCE for 2 hr. Each point represents the mean \pm SE for six rats. Percentage uptake of the inhaled dose was determined after 1, 3, 5, 10, 15, and 20 min and at 10-min intervals thereafter.

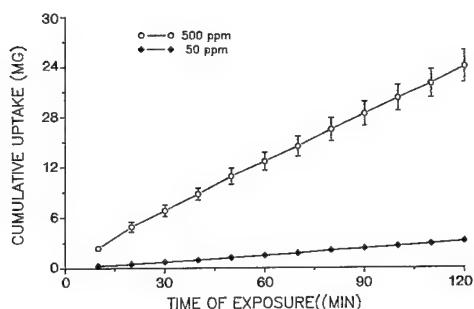


FIG. 5. Cumulative uptake of TCE during inhalation exposures. Rats inhaled 50 or 500 ppm TCE for 2 hr. The quantity of inhaled TCE retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled TCE concentrations. Each point represents the mean \pm SE for six rats.

els in the blood and breath of laboratory animals and humans. It might be anticipated that TCE would behave similarly to other relatively water-insoluble gases. This proved to be the case in the current study. As TCE is a small, uncharged, lipophilic molecule, it is readily absorbed across membranes of the pulmonary capillary bed into the systemic circulation. The net rate of transfer from alveoli to blood is initially very rapid, but becomes progressively slower as the chemical accumulates in the blood and tissues. The approach to equilibrium in the blood and exhaled breath is quite rapid, indicative of TCE's relatively low solubility in blood and the slow perfusion of adipose tissue, the major site of deposition of the chemical. These processes are reflected by the time-course of systemic uptake of TCE, where percentage uptake decreases over time from $\geq 95\%$ at the beginning of exposures to relatively constant levels of 69–71% at near steady-state. Studies in humans reveal lower uptake of inhaled TCE, with values ranging from 44 to 58% (Bartonecek, 1962; Astrand and Ovrum, 1976; Monster *et al.*, 1976, 1979). The greater percentage uptake in rats can be attributed in part to a difference in blood:air partition coefficients, in that values for the rat are 2½ to 3 times higher than for humans (Sato *et al.*, 1977; Gargas *et al.*, 1989). Other con-

tributing factors to the species difference likely include the higher respiratory rate and cardiac output of the rat, as well as its greater apparent capacity to metabolize TCE.

Sequential measurements of TCE uptake during the 2-hr inhalation sessions made it possible to accurately monitor the cumulative uptake (i.e., quantity retained in the body, or absorbed dose) of the chemical. There was a cumulative uptake of 8.4 mg/kg in rats inhaling 50 ppm TCE for 2 hr. When adjusted for exposure concentration and duration, this value is about four times the cumulative uptake reported by Monster *et al.* (1979) in humans inhaling 70 ppm TCE for 4 hr. Thus, rats received a substantially greater systemic dose of TCE on a mg/kg bw basis than do humans at equivalent inhaled concentrations.

Findings in the current study indicate that the rat's capacity to assimilate and metabolize TCE is exceeded during the course of the 2-hr, 500 ppm exposure. Although exhaled breath levels of TCE were directly proportional to the inhaled concentration, arterial blood levels rose 25- to 30-fold with the 10-fold increase in exposure. The blood levels of the 500 ppm animals progressively increased over the 2-hr period, rather than approaching equilibrium as was the case at 50 ppm. Stott *et al.* (1982) saw evidence of metabolic saturation in rats exposed for 6 hr to 600 ppm [^{14}C]TCE. Metabolic saturation was manifest by a decrease in metabolism and increase in exhalation of TCE, when the exposure level was increased from 10 to 600 ppm. Filser and Bolt (1979) calculated the saturation point for TCE metabolism to be 65 ppm on the basis of indirect vapor uptake studies in male rats. Andersen *et al.* (1987), also utilizing data from gas uptake experiments in male rats, determined the V_{\max} for TCE to be 11 mg/kg/hr. In the present study, the 10-fold increase in inhaled concentration (i.e., 50 to 500 ppm) resulted in an 8.7-fold increase in cumulative uptake, or total absorbed dose. Thus, metabolic saturation apparently commenced during the course of exposure, when there had

been systemic uptake of a finite quantity of TCE.

The capacity to metabolize TCE has been demonstrated by other investigators to be species-dependent. The metabolic saturation observed by Stott *et al.* (1982) in rats inhaling 600 ppm TCE for 6 hr was not seen at this exposure level in mice. The dose of TCE required to produce metabolic saturation in humans has not been clearly defined. Astrand and Ovrum (1976) saw no evidence of metabolic saturation in men inhaling 100 or 200 ppm TCE for up to 2 hr, in that percentage uptake was constant and absorbed dose was directly proportional to the inhaled concentration. Ikeda *et al.* (1972) reported that urinary concentrations of total trichloro compounds and trichloroethanol in occupationally exposed workers were proportional to inhaled concentrations of up to 175 ppm, but that there was a relative decrease in trichloroacetic acid at exposures above 50 ppm.

The major routes of elimination of TCE are metabolism and exhalation of the parent compound. The elimination of TCE in the exhaled breath generally paralleled elimination of the chemical from the bloodstream of rats in the present investigation. This pattern of elimination of TCE in the blood and breath is also typically seen in humans, although clearance is prolonged (Sato *et al.*, 1977; Nomiyama and Nomiyama, 1974). Despite species differences in TCE kinetics, comparable exhaled breath levels have been observed in rats and humans postexposure. Accounting for differences in exposure concentration, the postexposure exhaled breath levels of TCE from several studies in humans (Kimmerle and Eben, 1973a; Stewart *et al.*, 1974; Monster *et al.*, 1979) were similar in magnitude to the values of TCE eliminated in the exhaled breath of rats following inhalation exposure in the current investigation. Stewart *et al.* (1974), for example, measured concentrations of 0.70 and 0.28 ppm TCE in the exhaled breath of human subjects 30 and 120 min after termination of a 3-hr, 20 ppm inhalation exposure. Assuming a linear scaledown of the 50 ppm data from

the current study, the TCE concentrations in the expired air of rats at these two time-points would be 0.92 and 0.28 ppm, respectively. Such a similarity in the magnitude of exhaled breath levels in humans and rats was also noted recently for 1,1,1-trichloroethane (Dallas *et al.*, 1989). The rat blood:air partition coefficient for each VOC is significantly higher than that for humans (Gargas *et al.*, 1989). Although this difference alone would result in greater respiratory elimination of the VOCs by humans, it is apparently offset by other factors, including the higher respiratory and circulatory rates of the rat.

PB-PK models for TCE have been developed by several groups of investigators. Sato *et al.* (1977) formulated a PB-PK model for respiratory exposure of humans to TCE. The model included three compartments, with intercompartment exchange of TCE governed solely by intertissue diffusion. Metabolic and respiratory excretion were assumed to occur in the richly perfused tissue compartment. Fernandez *et al.* (1977) constructed a more complete PB-PK model, which accurately predicted respiratory elimination of TCE and cumulative urinary excretion of TCE metabolites in humans. This model included the three compartments of Sato *et al.* (1977), as well as a liver compartment with blood-flow-limited metabolism and a lung compartment for respiratory absorption and elimination of TCE. Andersen *et al.* (1987) used a PB-PK model analogous to that of Ramsey and Andersen (1984) to predict the influence of competitive metabolic inhibition on uptake of inhaled TCE in rats. Fisher *et al.* (1989) subsequently modified the Ramsey and Andersen (1984) model to simulate the kinetics of TCE and trichloroacetic acid in the pregnant rat following inhalation and ingestion of TCE. Additional compartments (i.e., mammary tissue, placenta, and fetus) were incorporated into the model, and allowance was made for certain physiological changes which occur during pregnancy. The PB-PK model of Fisher *et al.* (1989) provided a good representation of TCE and trichloroacetic acid levels mea-

sured experimentally in maternal and fetal blood at a limited number of times postexposure. This model has been extended recently to predict the kinetics of TCE and trichloroacetic acid in lactating rats and nursing pups (Fisher *et al.*, 1990).

The PB-PK model used in the current investigation accurately predicted the time-courses of TCE concentrations in the blood and exhaled breath of rats during and following inhalation exposure to 50 and 500 ppm TCE. The model is similar to those of Ramsey and Andersen (1984) and Angelo and Pritchard (1984). Our PB-PK model differs in that it includes a separate lung tissue compartment and a lung:alveolar mass transfer coefficient, which describes the bidirectional transfer of TCE across the alveolar membrane. It is only necessary to alter the experimentally determined inhaled concentration and minute volume in order to obtain simulations of TCE kinetics under different inhalation exposure scenarios. Metabolic saturation, manifest by the progressive, disproportionate increase in blood levels in the high-dose (i.e., 500 ppm) animals, was accurately forecast. There was also good agreement between predicted and observed blood and breath levels during most of the postexposure period. Previous investigators, including Fisher *et al.* (1989), have had the use of very limited experimental data sets for assessing the precision of their model predictions.

Health risk assessments of VOCs such as TCE require a careful selection of the measure of dose. Areas under blood and tissue concentration versus time curves have been advocated as logical measures of target organ dose (Andersen, 1987). The most appropriate chemical species to measure for TCE depends upon which toxic effect is of interest. TCE appears to be primarily responsible for CNS depression and cardiac arrhythmias. As it is unclear which metabolite(s) should be used as dose measures, or surrogates for TCE-induced cytotoxicity and mutagenicity/carcinogenicity, the amount of reactive intermediate (i.e., toxicologically effective dose) has been equated to the total

amount of TCE metabolized by the liver (Bruckner *et al.*, 1989). The NRC (1986) applied the PB-PK model of Ramsey and Andersen (1984) to calculate the toxicologically effective dose formed in the liver of rats during TCE inhalation exposure. Bogen (1988) has more recently applied the model of Ramsey and Andersen (1984) to predict relationships between the administered dose and the toxicologically active, or metabolized dose of TCE in humans. Comprehensive TCE time-course data sets, however, have not been available for rigorous validation of model predictions. The next logical step in this direction will be to obtain TCE/metabolite tissue concentration versus time data sets through direct measurement studies.

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APPENDIX B

Chen, X.M., Dallas, C.E., Muralidhara, S., Srivatsan, V., and Bruckner, J.V. "Analyses of volatile C₂ haloethanes and haloethenes in tissues: sample preparation and extraction." *Journal of Chromatography* **612**: 199-208 (1993).

Analyses of volatile C₂ haloethanes and haloethenes in tissues: sample preparation and extraction

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ABSTRACT

A tissue extraction procedure was developed which minimized loss of readily volatilizable compounds for subsequent quantification by headspace gas chromatography, and evaluated for perchloroethylene (PER), 1,1,1-trichloroethane, 1,1,2,2-tetrachloroethane, and 1,1,2-trichloroethylene. Of the procedures evaluated, joint isoctane and saline tissue homogenization had the most efficient recovery, ranging from 73 to 104% for the four halocarbons from seven different rat tissues. PER concentrations were also determined in tissues of rats following *in vivo* halocarbon administration. Recovery did not appear to be tissue-dependent, but did vary somewhat with test chemical, with the least volatile, most lipophilic compounds exhibiting the highest recovery.

INTRODUCTION

Short-chain aliphatic halogenated hydrocarbons (halocarbons) are a class of volatile organic compounds (VOCs) of increasing concern, due to their widespread occurrence as environmental contaminants and the potential risks they pose to health. Exposure to halocarbons can result in toxic injury of a number of organ systems in animals and humans. Central nervous system (CNS) dysfunction results from overexposure to most halocarbons and other VOCs [1,2]. CNS depressant effects have been directly correlated with the concentration of hydrocarbons in the brain [3,4]. Significant liver and kidney damage can be caused by certain halocarbons [5–7], while some members of this chemical class are carcinogenic

in different organ systems in animals [8–10].

Pharmacokinetic studies of halocarbons are needed in order to elucidate target organ uptake, deposition, and elimination of the chemicals. The magnitude of toxic effect in an organ is, of course, dependent upon the amount of chemical present in the tissue. Pharmacokinetic studies conducted to date have primarily involved measurement of concentrations of halocarbons in blood and exhaled breath [11–15]. The limited (*i.e.*, at a single time-point) tissue measurements conducted in some of these studies employed ¹⁴C-labeled halocarbons. Measurement of total radioactivity does not delineate between the parent compound, metabolites, and ¹⁴C which has entered the body's carbon pool. There have been a limited number of investigations, in which time-courses of tissue deposition of inhaled hydrocarbons have been delineated [3,4,16–18]. In these studies, the tissues were extracted with a solvent and the parent compounds quantified by gas chromatography (GC) or GC-mass spectral

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analysis. No reports of the time-course of uptake and elimination of halocarbons in tissues were found in the literature.

A variety of approaches have been used for analysis of VOCs in blood and tissues. One technique is to simply inject blood and tissue homogenates directly into a GC apparatus [16,19–21]. Major drawbacks of direct injection of biological materials are that the materials cause matrix interferences and that contamination markedly shortens the GC column's lifetime. Solvent extraction is a widely used approach for measuring concentrations of VOCs in blood and tissues. An aliquot of the solvent may be directly injected into the GC column [17,18,22,23]. Since this method typically involves a one-step extraction of the VOC with the solvent, limited sensitivity and interference by other lipophilic compounds can be problematic. In order to circumvent these difficulties, more complex procedures have been employed. One entails evaporation of the solvent extract and trapping of the VOC analyte on a Tenax column [24]. Another involves heating biological samples within a purging device [25–29], with subsequent retention of the analyte on an adsorbent such as Tenax. Such approaches are technically difficult and time-consuming. Headspace analysis has proven to be a sensitive and more efficient means of measuring VOC concentrations in blood samples [14,15,30,31]. No one, however, appears to have reported a suitable technique for quantification of halocarbons or other VOCs in tissues.

In light of the foregoing, it is apparent that there is a need for a rapid, sensitive analytical procedure for reliably measuring the concentrations of halocarbons and other VOCs in different tissues. The overall objective of this project was to adapt the headspace technique previously used for analysis of blood samples for measurement of C₂ halocarbons in different tissues. A major focus of the work was development of a procedure for conservation of the analyte (*i.e.*, minimization of loss by volatilization) during preparation and extraction of the tissue samples. Two C₂ haloalkanes and two C₂ haloalkenes were employed, in order to assess the utility of the proce-

dure for extraction and subsequent analyses of VOCs with different physicochemical properties.

EXPERIMENTAL

Test chemicals and apparatus

1,1,2,2-Perchloroethylene (PER), of 99% purity, and 1,1,2,2-tetrachloroethane (TET), of 97% purity, were obtained from Aldrich (Milwaukee, WI, USA). 1,1,1-Trichloroethane (TRI), of 99% purity, 1,1,2-trichloroethylene (TCE), of 99% purity, and isoctane, of 99.98% purity, were purchased from J. T. Baker (Phillipsburg, NJ, USA). A Sigma Model 300 gas chromatograph equipped with a HS-6 headspace sampler (Perkin Elmer, Norwalk, CT, USA) and a Model 5890 gas chromatograph equipped with a 19395A headspace sampler (Hewlett Packard, Avondale, PA, USA) were used for the analysis of halocarbons. Both the gas chromatographs were equipped with an electron-capture detector. Analyses were carried out on stainless-steel columns (182 cm × 0.317 cm I.D.) packed with 10% FFAP (Alltech Assoc., Deerfield, IL, USA), 3% SP 1000 (Supelco, Bellefonte, PA, USA), or 3% OV-17 (Alltech Assoc.). Tissues were homogenized using the Ultra-Turrax SDT homogenizer (Tekmar, Cincinnati, OH, USA).

Tissue homogenization and extraction procedures

Twelve-week-old male Sprague–Dawley rats were obtained from Charles River Labs. (Raleigh, NC, USA). After two to three weeks, groups of four or eight animals (body weight = 325–375 g) were anesthetized with diethyl ether. Blood samples (1 ml) were withdrawn by closed chest cardiac puncture. Portions (0.5–1 g) of liver, kidney, brain, heart, lung, perirenal fat, and skeletal muscle were removed and placed onto ice. Each tissue was spiked with PER, TET, TCE, or TRI, by injection of 4 µl/g tissue of a solution containing 1 mg halocarbon per ml isoctane. Two homogenization approaches were evaluated using PER. In the first, the tissues were immediately transferred after halocarbon injection to previously chilled 20-ml glass vials containing 4 ml of ice-cold saline. The tissues were allowed to

remain in the tightly capped vials for approximately 30 min, before being homogenized for an established time interval with a Tekmar tissue homogenizer. These times were kept as brief as possible in order to minimize volatilization of the halocarbons. Brain, liver, and fat were the most easily homogenized, requiring only 3–4 s. Kidney, lung and heart required 5–8 s. Skeletal muscle was the most difficult to homogenize, in that it required 20 s. Isooctane (8 ml) was added to the homogenates, which were then vortex-mixed for 30 s and centrifuged at 1800 g for 10 min at 4°C in capped vials. An aliquot of the isooctane layer was transferred to capped vials for headspace analysis. In the second approach, tissues were immediately transferred after halocarbon injection to previously chilled 20-ml glass vials containing 2 ml of ice-cold saline and 8 ml of isooctane. Each tissue was then homogenized, vortex-mixed, and centrifuged as before. An aliquot of the isooctane layer was taken for headspace analysis. Only the latter approach (*i.e.*, isooctane homogenization) was subsequently used for determination of TCE, TRI, TET, and PER in tissues, except in the aliquot volume study.

Isooctane aliquot volume study

An experiment was conducted to determine the effect of aliquot volume on the linearity of halocarbon quantification. As in other *in vitro* experiments, a Hamilton gas-tight syringe was used to inject the chemical into the center of the tissue cubes. A 4- μ l volume of PER was injected into samples of blood and each of the seven tissues. The tissues were homogenized in saline and subsequently extracted with isooctane as described previously. Aliquots (5–100 μ l) of isooctane extract were withdrawn with a pipet and transferred to 8-ml headspace vials. These vials were capped immediately with rubber septa and a spring washer and crimped to insure an airtight seal. Each sample vial was then placed into the autosampler unit of the gas chromatograph.

Headspace gas chromatographic techniques

For all the experiments with PER, the GC operating conditions were: headspace sampler tem-

perature, 90°C; injection port temperature, 200°C; column temperature, 110°C; detector temperature, 400°C; column packing, 10% FFAP; flow-rate for argon–methane carrier gas, 60 ml/min. Operating conditions for TET were: headspace sampler temperature, 100°C; injection port temperature, 200°C; column temperature, 150°C; detector temperature, 400°C; column packing, 3% OV-17; flow-rate for argon–methane carrier gas, 60 ml/min. Operating conditions for TRI and TCE were: headspace sampler temperature, 55°C; injection port temperature 150°C; column temperature, 60°C; detector temperature, 400°C; column packing, 3% SP 1000. Except for the isooctane aliquot volume study, all analyses were conducted using a 20- μ l aliquot of isooctane in the 8-ml headspace vials. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 s with the carrier gas, and injected automatically by a solenoid-controlled mechanism into the GC column.

The aforementioned conditions resulted in vaporization of the halocarbons in the sample vials, since each VOC was heated to a temperature slightly below its boiling point, and the vial subsequently pressurized and vented into the GC. An experiment was conducted to assess the influence of heating time on quantification of PER. Heating sample vials 5 min or longer resulted in a constant GC detector response (*i.e.*, area under the curve) for a series of known quantities of PER (data not shown).

As saline was utilized in the tissue homogenization procedures, an experiment was conducted to determine whether the presence of saline influenced standard curves. PER was incorporated into four different solvent systems: 8 ml isooctane; 2 ml saline + 8 ml isooctane; 4 ml saline + 8 ml isooctane and 4 ml saline + 8 ml isooctane homogenized for 30 s. Each solution was vortex-mixed for 30 s. Aliquots of 1–25 μ l of the isooctane layer, equivalent to 1–25 ng PER, were subjected to GC headspace analysis. Standard curves were generated on the basis of the GC peak area plots. The slopes, intercepts, and correlation coefficients of the curves were compared. As described in the Results section, the standard curves

did not vary significantly from one solvent system to another. Therefore, the simplest system was subsequently employed for preparation of standard solutions of PER, TET, TCE, and TRI (*i.e.*, an appropriate amount of halocarbon was dissolved in isoctane alone). Standard curves for each compound were generated the same day that sample analyses were performed, using the same analytical conditions.

The limit of detection of the GC assay was determined by the method described by MacDougall and Chummett [32]. A signal-to-noise ratio of 3 or greater was considered as the limit of detection. The presence of background noise and any interfering peaks was assessed in air and in isoctane samples. Neither was observed, as the detector baseline was consistently stable. The limit of detection for TRI, TCE, PER, and TET was found to be 1 ng. This amount is equivalent to 8.4, 8.5, 6.7, and 6.6 parts of chemical per billion parts of air for TRI, TCE, PER, and TET, respectively.

In vivo tissue measurements

The concentration of PER was determined in tissues of rats following intra-arterial administration of the compound. Male Sprague–Dawley rats of 325–375 g from Charles River Labs. were surgically implanted with an indwelling carotid artery cannula. The cannula exited the body behind the head, so the animal could not disturb the cannula, but have freedom of movement. Food was withheld during an 18-h recovery period before PER administration. PER was incorporated into undiluted polyethylene glycol 400, and a dose of 10 mg PER/kg body weight injected as a single bolus into the arterial cannula. Each animal was anesthetized with diethyl ether 1 h after dosing, and blood taken by closed-chest cardiac puncture. Portions (1 g) of liver, kidney, brain, heart, lung, perirenal fat, and skeletal muscle were excised within 2.5 to 3 min from each animal and immediately placed into chilled vials containing 2 ml of saline and 8 ml of isoctane. The samples were processed for PER analysis using the isoctane homogenization procedure described previously.

Statistics

Comparisons of the percentage recovery of PER from tissues and blood, using the two homogenization procedures, were made using Student's *t*-test. A two-way analysis of variance was utilized to assess the significance of variances among standard curves for the different saline–isoctane mixtures. Values were considered significantly different at $p < 0.05$. The inter-assay variation had a coefficient of variation that did not exceed 12%, and the intra-assay coefficient of variation was less than 10% for all compounds tested.

RESULTS

Results of the study on the effect of isoctane aliquot volume on the linearity of halocarbon quantification are presented in Fig. 1. A very similar pattern was observed for all tissues studied. The quantity of PER increased linearly with increasing aliquot volume up to 25 μ l. Use of larger aliquots of isoctane (*i.e.*, 50 and 100 μ l) did not result in any further increase in the amount of measurable PER.

Recovery values (%) obtained using the saline and isoctane homogenization approaches for PER are contrasted in Table I. Recoveries were quite good with both procedures, in that values ranged from approximately 72 to 104%. Recoveries of PER from kidney, fat, lung, muscle, and brain were significantly higher when the tissues were homogenized directly in isoctane. Recovery of PER from liver, heart, and blood did not differ significantly for the two procedures.

Recoveries (%) of PER, TET, TCE, and TRI from spiked tissues, utilizing the isoctane homogenization procedure, are tabulated in Table II. Recovery of TET was generally higher than was the case for the other three chemicals. Recovery of TCE was generally the lowest of the four chemicals, with no mean values exceeding 88% for any tissue. Indeed, the lowest recovery of TET (*i.e.*, from fat) was greater than the highest recovery of TCE (*i.e.*, from muscle). The mean recoveries of PER, TET, and TRI from fat were quite similar (within 2%). TCE recovery (73%)

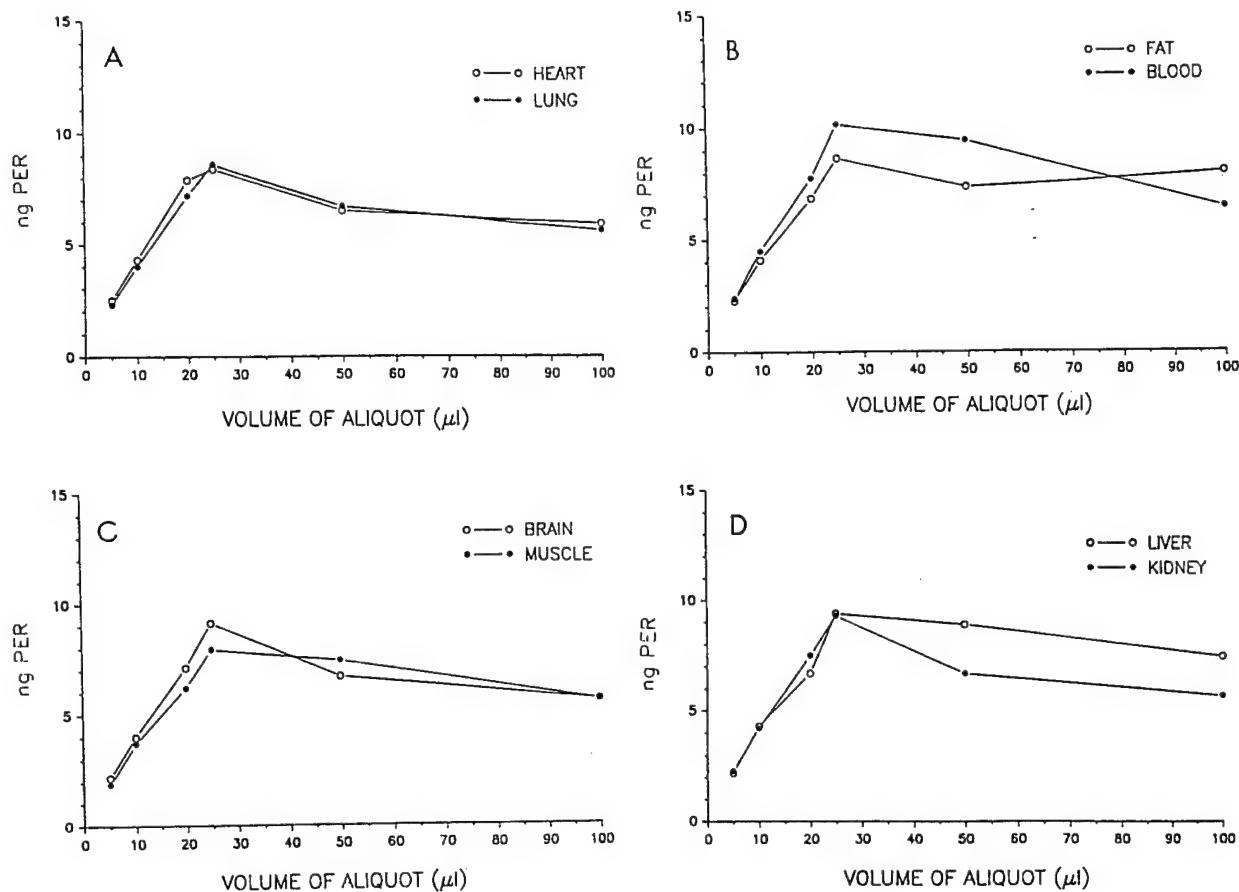


Fig. 1. Effect of isooctane aliquot volume on linearity of PER content in samples from heart and lung (A), fat and blood (B), brain and muscle (C), and liver and kidney (D). Aliquot volumes of 5, 10, 20, 25, 50, and 100 μ l were withdrawn from the organic phase of the tissue homogenate and analyzed by GC headspace analysis.

from fat was the lowest for any chemical from any tissue. The range in values for the different tissues was the smallest for TRI (*i.e.*, less than 6%) and the largest for TCE (*i.e.*, 14.9%). The recovery of the four volatile chemicals from tissues with the shortest homogenization time (*i.e.*, liver, fat, and brain) was not substantially different from that from other tissues. Unexpectedly, there was relatively high recovery of all four halocarbons from skeletal muscle, the tissue requiring the longest homogenization time. No tissue consistently exhibited higher or lower recovery values for any of the four chemicals.

Standard curves for PER standards, prepared using four different solvent and saline mixtures, are shown in Fig. 2. The linear regression equa-

tions were determined to be $y = 25.9x - 13.3$ for 8 ml isooctane, $25.9x - 8.4$ for the 2 ml saline-8 ml isooctane mixture, $y = 25.8x - 8.0$ for the 4 ml saline-8 ml nonhomogenized isooctane mixture, and $y = 25.2x - 3.6$ for the 4 ml saline-8 ml homogenized isooctane mixture. For all of the data considered together, the linear regression equation was $y = 24.3x - 6.9$. Thus, there was no statistically significant difference between the regression equations for the four solvent systems. Therefore, neither the presence of saline nor homogenization significantly affected standard curves for PER.

Concentrations of PER, measured in tissues of rats 1 h following intra-arterial administration of a single 10 mg/kg dose of PER, are shown in Ta-

TABLE I
EFFECT OF HOMOGENIZATION PROCEDURE ON THE RECOVERY OF PER

Each value represents the mean \pm S.E. for recovery of 1,1,2,2-tetrachloroethylene (PER) from tissues of eight rats for isoctane homogenization and four rats for saline homogenization.

Sample	Recovery (%)	
	Saline homogenization	Isooctane homogenization
Liver	95.5 \pm 9.9	89.6 \pm 3.1
Kidney	69.0 \pm 4.9 ^c	86.7 \pm 1.4 ^c
Fat	73.8 \pm 4.9 ^a	88.2 \pm 2.7 ^a
Heart	75.8 \pm 10.3	81.2 \pm 1.2
Lung	78.8 \pm 7.1 ^b	99.1 \pm 2.3 ^b
Muscle	80.3 \pm 6.3 ^a	98.5 \pm 2.9 ^a
Brain	72.3 \pm 5.7 ^b	88.6 \pm 2.0 ^b
Blood	104.8 \pm 5.5	95.4 \pm 4.1

^a Significant difference between procedures at $p < 0.05$.

^b Significant difference between procedures at $p < 0.01$.

^c Significant difference between procedures at $p < 0.001$.

ble III. PER levels in the fat were an order of magnitude or more higher than in any other tissue sampled. The concentrations of PER measured in the liver, kidney, heart, and lung were relatively consistent (*i.e.*, within 25% of each other). Brain levels of PER were approximately two-

fold higher than in these organs, while the blood and skeletal muscle exhibited the lowest concentrations.

DISCUSSION

GC techniques are routinely used to determine levels of VOCs in environmental and biological samples. Wallace *et al.* [33] utilized GC purge-and -trap techniques to conduct large-scale surveys of human exposure to VOCs in drinking water, indoor and outdoor air. GC purge- and -trap techniques have also been used successfully to measure concentrations of VOCs in human blood [27,29,30,34], milk [26,27], and urine [27]. These assays are precise and quite sensitive, as many of the investigators employed GC-mass spectrometric computer analyses. Other investigators have used static GC headspace methods to quantify halocarbons and other VOCs in blood [14,15,30,31]. Such headspace analyses offer the advantages of speed and simplicity, such that large numbers of samples can be assayed daily using a gas chromatograph equipped with an autosampler. Although each of the aforementioned techniques generally work well for air and liquids, little attention has been devoted to adapting them for measurement of VOCs in solid tissues.

TABLE II

RECOVERY OF C₂ HALOALKANES AND HALOALKENES FROM BLOOD AND TISSUES

Values represent the mean \pm S.E. for measurement in spiked tissues taken from eight rats. Each spiked tissue was homogenized in 8 ml ice-cold isoctane and 2 ml saline, vortex-mixed, centrifuged at 4°C, and an aliquot of the isoctane assayed by headspace GC as described in Experimental.

Sample	Recovery (%)			
	PER	TET	TCE	TRI
Liver	89.6 \pm 3.1	96.1 \pm 2.1	86.0 \pm 1.3	91.3 \pm 4.0
Kidney	86.7 \pm 1.4	97.9 \pm 1.5	86.9 \pm 1.6	88.4 \pm 4.9
Fat	88.2 \pm 2.7	89.9 \pm 1.4	73.0 \pm 1.5	88.7 \pm 2.2
Heart	81.2 \pm 1.2	98.1 \pm 1.4	85.9 \pm 3.5	89.6 \pm 2.4
Lung	99.1 \pm 2.3	96.1 \pm 0.8	80.0 \pm 0.7	89.7 \pm 1.9
Muscle	98.5 \pm 2.9	97.4 \pm 1.1	87.9 \pm 1.7	87.6 \pm 4.9
Brain	88.6 \pm 2.0	100.3 \pm 3.1	80.7 \pm 2.8	87.6 \pm 4.6
Blood	95.4 \pm 4.1	97.3 \pm 2.1	85.9 \pm 2.0	85.5 \pm 3.7

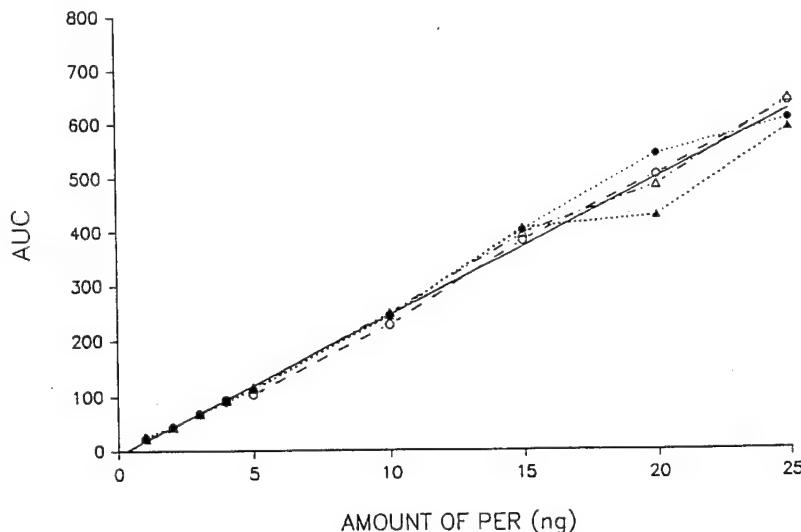


Fig. 2. Standard curves for PER using four solvent and saline combinations and homogenization: (○) 8 ml isoctane; (◆) 8 ml isoctane + 2 ml saline; (△) 8 ml isoctane + 4 ml saline and (▲) 8 ml isoctane + 4 ml saline, homogenized for 30 s. The detector response, presented here as area under the curve (AUC), is plotted against amount of PER. The regression equation for the data presented here is $y = 24.3x - 6.9$.

A practical technique for processing and extracting C₂ halocarbons from tissues for subsequent GC headspace analyses is reported here. Several approaches for determination of VOCs in tissues have been employed previously, with limited success. Direct injections of tissue homog-

enates or solvent extracts of homogenates have a number of inherent problems, including loss of the VOC by volatilization, GC column contamination, interference by biological matrices and lipophilic macromolecules, and limited sensitivity. One method for measuring toluene in blood and tissues involved extraction with methanol, selective adsorption onto Tenax, and desorption from the Tenax with heat into a gas chromatograph [24]. A significant problem in measuring VOCs in solid tissues is volatilization of the analyte during tissue processing. Peterson and Bruckner [24] attempted to overcome this difficulty by crushing the tissues with a rod under methanol within a closed container. This technique was reasonably successful (e.g., 73 and 92% recovery of toluene from liver and brain, respectively), but recoveries from other tissues were limited by incomplete maceration and escape of toluene from the maceration-extraction container. This technique was also labor-intensive and time-consuming, as was a purge- and -trap method described by Lin *et al.* [28] for measuring 1,1- and 1,2-dichloroethylene (1,2-DCE). The procedure of Lin *et al.* [28] involved thermal desorption of halocarbons from previously

TABLE III
TISSUE CONCENTRATIONS OF PER IN RATS FOLLOWING *IN VIVO* EXPOSURE

Each animal was sacrificed 1 h after intra-arterial administration of 10 mg 1,1,2,2-tetrachloroethylene (PER) per kg body weight.

Tissue	PER concentration ^a ($\mu\text{g/g}$)
Liver	2.4 \pm 0.5
Kidney	2.7 \pm 0.4
Fat	48.1 \pm 4.1
Heart	3.0 \pm 0.2
Lung	2.3 \pm 0.2
Muscle	1.9 \pm 0.2
Brain	4.7 \pm 0.4
Blood	1.3 \pm 0.1

^a Values represent the mean \pm S.E. for four rats.

minced tissues within a purging device. The chemicals were subsequently trapped on a Tenax column and desorbed with heat into a gas chromatograph. Mean recovery values for 1,2-DCE from liver, kidney, brain, and adipose tissue were 60, 53, 63 and 93%, respectively [28]. The purge-and-trap technique of Pellizzari and co-workers [27,35] also resulted in low recovery (*i.e.*, 13–80%) of a series of halocarbons from adipose tissue. The method involved transfer of 5-g portions of frozen adipose tissue to a 100-ml round-bottom purging flask maintained in an ice bath, addition of the halocarbon dissolved in distilled water, and maceration with a Virtis tissue homogenizer. The flask's contents were then heated to 50°C, stirred and purged with helium for 30 min, in order to transfer the analyte to a Tenax column. These researchers [27,35] attributed their low recovery values and marked inter-sample variability to halocarbon losses during tissue maceration and transfer, as well as retention of the analyte by complex matrices and lipophilic compounds. In contrast, the technique presented in the current paper is quite efficient, in that it involves a homogenization-extraction step and the relative ease and speed of GC headspace analysis. The method was also sensitive (*i.e.*, limit of detection = 1 ng) and efficient, in that recoveries of four different halocarbons from a variety of tissues were quite high (*i.e.*, 73–104%) and consistent (*i.e.*, highest S.E. = 4.9%).

An important factor in the present procedure was the limitation in the volume of aliquot that could be employed in the headspace vials. As standard curve measurements were no longer linear at volumes above 25 µl, a 20-µl aliquot was selected for subsequent use. There was a statistically higher recovery from most tissues when using isoctane homogenization than when using saline homogenization. It appears that homogenization of tissues in an aqueous solution (*i.e.*, saline), with subsequent extraction into isoctane, provided more opportunity for loss of the volatile chemicals through evaporation than did the single step of homogenization in isoctane. Isooctane proved to be superior to a variety of other organic solvents for extraction of all four

halocarbons (unpublished data). Other solvents that were employed included methanol, ethyl acetate, *n*-hexane, cyclohexane, and toluene. Some solvents (*e.g.*, ethyl acetate) worked well for one halocarbon, but not for others. Isooctane provided the highest recovery without interfering peaks for all four halocarbons.

The applicability of this approach for analysis of C₂ halocarbons was demonstrated by its use with two haloalkanes and two haloalkenes with differing physicochemical properties. As would be expected, the relative volatility of the chemicals affected the recovery. The boiling points of TRI, TCE, PER, and TET are 74, 86.7, 121, and 140.7°C, respectively [36]. TET, the least volatile chemical, exhibited the highest recovery from most tissues. Recovery of PER was also relatively high from each tissue except the heart. It is noteworthy that PER is the most lipophilic of the halocarbons [37], and therefore should be most efficiently extracted by isoctane. TCE generally exhibited the lowest recovery values, as would be anticipated from its relatively high volatility and low lipophilicity. Recovery of TRI was unexpectedly high from most tissues. One would predict that TRI recovery should also be relatively low, since it was the most volatile and one of the least lipophilic of the four compounds studied.

A basic tenet of toxicology is that of the dose-response relationship (*i.e.*, the magnitude of toxic effect is a function of the administered dose). The concept of dose is now being refined, as it is recognized that the amount of chemical absorbed systemically (*i.e.*, the internal dose) can vary significantly with route of exposure, dosing vehicle and animal species. The blood level over time following exposure has been accepted historically as an index of internal dose, but it often may not accurately reflect concentration of chemicals at sites of action within tissues. Thus, the most logical and precise measures of dose are time integrals of target organ concentrations of bioactive chemicals [38]. Unfortunately, there are a paucity of tissue level *versus* time data sets for VOCs, due largely to the technical difficulties and the inordinate time involved in quantification of these highly volatile compounds in individual samples.

In order to derive appropriate time-course data, tissue concentrations must be measured sequentially during and post exposure in separate groups of animals, necessitating analysis of a large number of samples. A technique is presented here, which allows rapid processing and extraction of C₂ halocarbons from a variety of organs, for subsequent GC headspace analyses. By use of such a procedure, it should be possible to generate comprehensive tissue dose-time-course data to correlate with toxicity data. Recognition and utilization of such information can substantially reduce uncertainties inherent in toxicity and carcinogenicity risk assessments of halocarbons and other VOCs.

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APPENDIX C

Dallas, C.E., Chen, X.M., O'Barr, K., Muralidhara, S., Varkonyi, P., and Bruckner, J.V.
"Development of a physiologically based pharmacokinetic model for perchloroethylene using
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Development of a Physiologically Based Pharmacokinetic Model for Perchloroethylene Using Tissue Concentration-Time Data^{1,2}

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The tissue disposition of perchloroethylene (PCE) was characterized experimentally in rats in order to (1) obtain input parameters from *in vivo* data for the development of a physiologically based pharmacokinetic (PBPK) model, and (2) use the PBPK model to predict the deposition of PCE in a variety of tissues following inhalation exposure. For the derivation of model input parameters, male Sprague-Dawley rats received a single bolus of 10 mg PCE/kg body wt in polyethylene glycol 400 by ia injection through an indwelling carotid arterial cannula. Other male Sprague-Dawley rats inhaled 500 ppm PCE for 2 hr in dynamic exposure inhalation chambers. Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, perirenal fat, and blood were taken for up to 72 hr following ia injection, during the 2-hr inhalation exposure, and for up to 72 hr postexposure. Blood and tissue PCE concentrations were analyzed using a gas chromatography headspace technique. Following ia administration, the tissues exhibited similar terminal elimination half-lives ($t_{1/2}$). As comparable tissue $t_{1/2}$ s are consistent with a blood-flow-limited model, tissue:blood partition

coefficients were calculated for noneliminating compartments by division of the area under the tissue concentration-time curve (AUC) by the blood AUC. Liver PCE concentration versus time data were employed in the calculation of *in vivo* metabolic rate constants. A PBPK model was developed using these parameters derived from the ia data set and used to predict tissue PCE concentrations during and following PCE inhalation. Predicted tissue levels were in close agreement with the levels measured over time in the seven tissues and in blood. Tissue concentration-time data can thus provide valuable input for parameter estimation and for validation of PBPK model simulations, as long as independent *in vivo* data sets are used for each step. © 1994 Academic Press, Inc.

Tetrachloroethylene, or perchloroethylene (PCE), is a volatile halogenated hydrocarbon that is widely used as a synthetic intermediate and a general solvent in operations such as metal degreasing and dry cleaning. Because of its widespread use, PCE is commonly found as a contaminant of air and groundwater (ATSDR, 1993). PCE has been identified in samples from 714 of 1300 hazardous waste sites on the EPA's National Priorities List (HAZDAT, 1992). It has been estimated that 688,110 workers employed at 49,025 industrial facilities may be exposed to PCE (NOES, 1990). Occupational exposure limits have been based on PCE's ability to reversibly inhibit central nervous system functions. Inhalation of high concentrations of the halocarbon can also sensitize the myocardium to catecholamines, resulting in cardiac arrhythmias. Hepatotoxicity is seen rarely in workers, even those exposed to very high vapor levels (ATSDR, 1993). Of greater concern, particularly at environmental exposure levels, is the potential for PCE to cause cancer. Tumors have been reported in mice and/or rats in oral (NCI, 1977) and inhalation (NTP, 1986) carcinogenicity bioassays. Thus, there is considerable interest at present in risks PCE may pose to human health in both occupational and environmental settings.

Several groups of investigators have developed physiologically based pharmacokinetic (PBPK) models to simulate PCE disposition in laboratory animals and humans (Gu-

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beran and Fernandez, 1974; Chen and Blancato, 1987; Ward *et al.*, 1988; Bois *et al.*, 1990). In PBPK models, the body is divided into anatomical compartments representing individual organs or groups of organs which share a common characteristic. A mass balance differential equation is written for each compartment in the model, based upon anatomical and physiological parameters (i.e., tissue volumes and blood flow rates) for the test species, and upon physicochemical (i.e., partition coefficients) and biochemical parameters (i.e., metabolic rate constants) for each chemical (Gerlowski and Jain, 1983). Solution of the set of mass balance differential equations generates chemical concentrations as a function of time in each compartment/tissue.

Assessment of the accuracy of predicted chemical concentrations over time in tissue compartments is important in determining PBPK model reliability. Efforts to establish the accuracy of PBPK model simulations of systemic uptake and elimination of volatile organic compounds (VOCs) have largely involved VOC blood and exhaled-breath data (Andersen *et al.*, 1987; Ward *et al.*, 1988; Reitz *et al.*, 1988; Fisher *et al.*, 1989). Blood levels over time, as a measure of bioavailability, have classically been used as an index of the level of chemical in the body and are therefore representative of tissue levels and target organ effects. This assumption can be misleading in that blood concentrations may not be reflective of the concentration of chemical or active metabolite at the site of action in a target tissue. A more logical measure of target organ exposure might be the area under the tissue concentration versus time curve for the active chemical or the peak blood concentration, depending upon the chemical and its mechanism of action.

There has been very little published to date on the ability of PBPK models to accurately predict time integrals of tissue exposure to halocarbons and other VOCs, apparently due to a paucity of tissue concentration versus time data sets. This lack of data is largely due to the considerable time and effort required in such studies and to technical problems with quantitation of the volatile chemicals in solid tissues. The only tissue data Reitz *et al.* (1988) had to use in testing their 1,1,1-trichloroethane (TRI) model were levels of radioactivity measured by Schumann *et al.* (1982) in the liver and fat of mice and rats at the termination of 6-hr inhalation exposures to [¹⁴C]TRI. Measurement of radioactivity, of course, does not distinguish between parent compound, metabolites, and ¹⁴C which has entered the carbon pool. It should also be recognized that VOCs are very rapidly absorbed from the lungs (Dallas *et al.*, 1983, 1989) and gastrointestinal tract (D'Souza *et al.*, 1985; Putcha *et al.*, 1986) into the bloodstream and transported to tissues. Manifestations of central nervous system effects (Bruckner and Peterson, 1981) and hepatocytotoxicity (Rao and Recknagel, 1968; Lowrey *et al.*, 1981; Luthra *et al.*, 1984) can occur within a few minutes of the onset of VOC expo-

sure. Thus, it is important to fully characterize entire time courses of tissue uptake and elimination of VOCs, particularly during the "critical early minutes" of exposure, when many events important in cytotoxicity and cellular dysfunction occur.

The present study was conducted to generate comprehensive tissue concentration versus time data sets to use for determination of *in vivo* input parameters and for validation of a PBPK model for PCE exposure of rats. The halocarbon was administered by ia injection and the resulting time-course data used to derive *in vivo* parameters including tissue: blood partition coefficients and metabolic rate constants. A PBPK model developed on this basis was then tested for its accuracy by comparison of simulations with observed tissue concentration-time data during and following inhalation exposure of rats to PCE.

METHODS

Adult male Sprague-Dawley rats (325–375 g), obtained from Charles River Laboratories (Raleigh, NC) were utilized in these studies. The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. Rats were housed in stainless steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow were provided *ad libitum*. The rats were used after at least a 7-day acclimation period, at which time they were approximately 12 weeks old. PCE exposures were initiated between 1000 and 1200 hr each day.

1,1,2,2-Perchloroethylene (tetrachloroethylene) of 99%+ purity was obtained from Aldrich Chemical Company (Milwaukee, WI). The purity of the chemical was verified by gas chromatographic analysis. A cannula was surgically implanted into the left common carotid artery of one group of rats. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg body wt of a mixture consisting of ketamine HCl (100 mg/ml):acepromazine maleate (10 mg/ml):xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v/v). The cannulas were protected from manipulation by exteriorizing and surgically taping them at the back of the neck. The cannulated animals were allowed complete freedom of movement during a 24-hr recovery period. All animals employed in the ia studies were freely moving and unanesthetized. PCE was given through the arterial cannula as a single bolus in a dose of 10 mg/kg body wt using polyethylene glycol 400 (1 mg/kg body wt) as a vehicle.

Additional groups of rats were exposed to 500 ppm PCE for 2 hr in 1.0-m³ Rochester-type constant/dynamic flow chambers. Air or nitrogen was passed through a glass dispersion flask with PCE at a constant rate and directed into the chamber influent air stream. A heating mantle was placed around the dispersion flask and narrow temperature limits maintained for continuity of volatilization. The entire halocarbon-generating system was enclosed in a specially fabricated safety box. The box was maintained under constant negative pressure during exposure sessions, as were the inhalation chambers. Exhaust air from the chambers and the generation box was vented through HEPA and activated charcoal filters so that the chemical was removed before release of effluent air to the environment.

The chambers were operated at flow rates of 7 to 15 ft³/min ($\frac{1}{4}$ to $\frac{1}{2}$ change of the chamber volume per min). A negative pressure of 20 to 50 mm Hg was maintained at all times during operation of the chambers. PCE concentrations in the chamber air were monitored by withdrawing 1-ml samples of air from the chamber and injecting them into a Tracor MT560 gas chromatograph (GC) equipped with a flame ionization detector (FID) (Tracor Instruments, Austin, TX). Characterization of the chamber test atmospheres verified that the PCE concentrations in the indi-

vidual wire-mesh cages, which comprised the breathing zone of the animal, were within 10% of the samples withdrawn for the chamber monitoring during the exposure. Standards were prepared in each of four 9-liter bottles, equipped with Teflon stoppers with needles from which air samples could be taken. Air samples were procured from the bottles and chambers with a gas-tight, 1-ml syringe and injected directly onto an 8-ft $\times \frac{1}{8}$ -in stainless steel column packed with 0.1% AT 1000 on GraphPak. Operating temperatures were: 150°C, injection port; 200°C, FID detector; 350°C, isothermal column operation.

In a typical exposure sequence, groups of five rats were placed into sets of wire-mesh exposure cages and positioned in the exposure chamber. Each animal was individually housed, so the animals could not limit their inhalation of the halocarbon vapors by burying their nose in one another's fur.

Groups of rats ($n = 4$) were anesthetized with ether and euthanized at the following times following ia dosing: 1, 5, 10, 15, 30, and 60 min; and 2, 4, 6, 12, 36, 48, and 72 hr. For inhalation exposures, sampling times for the groups of five rats were 15, 30, 60, 90, and 120 min during exposure; and 0.25, 0.5, 1, 2, 4, 6, 12, 24, 36, 48, and 72 hr postexposure. Blood samples were obtained by cardiac puncture of the left ventricle. Approximately 1-g samples of brain, liver, kidney, lung, heart, perirenal fat, and skeletal muscle were then quickly excised and placed into previously chilled 20-ml glass vials containing 2 ml of ice-cold saline and 8 ml of isoctane. Each tissue was then homogenized for an established time interval with a Ultra-Turrax SDT homogenizer (Tekmar Co., Cincinnati, OH). These times were kept as brief as possible in order to minimize volatilization of the halocarbon. Brain, liver, and fat were the most easily homogenized, requiring only 3–4 sec. Kidney, lung, and heart required 5–8 sec. Skeletal muscle was the most difficult to homogenize; it required 20 sec. The homogenates were then centrifuged at 1800g for 10 min at 4°C in capped vials. An aliquot of the isoctane layer was transferred to 8-ml headspace vials. These vials were capped immediately with rubber septa and a spring washer and crimped to insure an airtight seal. This tissue extraction procedure has been recently described in detail by Chen *et al.* (1993).

A Sigma model 300 GC equipped with a HS-6 headspace sampler and an electron capture detector (ECD) (Perkin-Elmer Co., Norwalk, CT) was used for the analysis of PCE in biological samples. Analyses were carried out using a stainless steel column (182 \times 0.317 cm) packed with 10% FFAP (Alltech Associates, Deerfield, IL). The GC operating conditions were: headspace sampler temperature, 90°C; injection port temperature, 200°C; column temperature, 110°C; ECD temperature, 400°C; flow rate for argon/methane carrier gas, 60 ml/min. Each sample vial was heated thermostatically to 90°C for 10 min in the GC autosampler unit, pressurized for 30 sec with the carrier gas and 20 μ l of volatilized isoctane, and PCE injected automatically into the GC column. The limit of detection for PCE was approximately 1 ng, or 6.7 parts of PCE per billion parts of air in the sample vial. The percentage recovery of PCE from spiked blood and tissue samples ranged from 81 to 99% (Chen *et al.*, 1993). Reproducibility, as reflected by SE values in this recovery experiment, was quite good. Thus the analytical technique proved to be a sensitive, reliable means of quantifying PCE in the large number of biological samples utilized in the current investigation.

The disposition of PCE during and following inhalation exposures in the rat was predicted using a PBPK model (Fig. 1), developed using tissue concentration-time data following ia administration. This model is similar to the model previously developed by Ramsey and Andersen (1984) for other VOCs in that it provides for inhalation and exhalation through a lung compartment, metabolism in a liver compartment, and blood-flow-limited organ representations. The current model differs primarily in that it includes a greater number of tissue compartments. Another difference is the use of a mass transfer coefficient in the lung compartment, which is based on the alveolar-permeability-area product for methylene chloride (Angelo and Pritchard, 1984). Values measured in our laboratory for cardiac output, tissue blood flows (Delp *et al.*, 1991), tissue volumes, and lipid

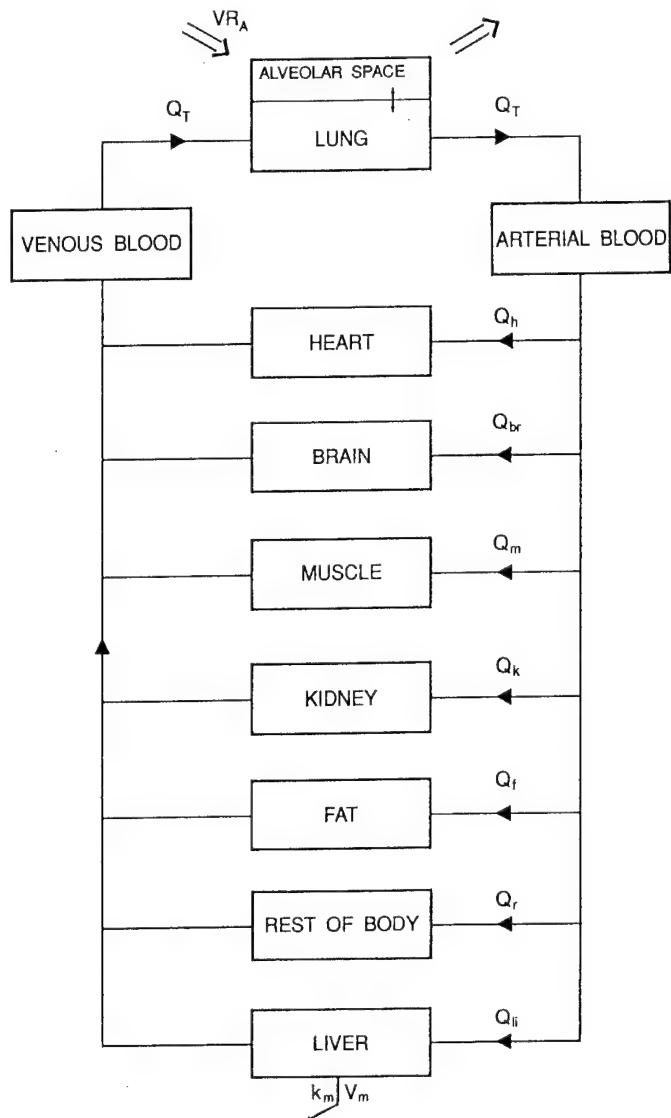


FIG. 1. Diagram of the physiological pharmacokinetic model used to simulate the disposition of PCE in blood and tissues during and following inhalation exposures in rats. The parameters used to describe the model are included in Table 1.

content (Manning *et al.*, 1991) in male Sprague-Dawley rats were employed (Table 1). Alveolar ventilation values for Sprague-Dawley rats inhaling 500 ppm PCE were determined in a companion study (Dallas *et al.*, 1994), where the airflow created by the animal's inspiration was recorded both during and following PCE exposure. For the current study using unrestrained animals, 70% of the minute volume was utilized as the alveolar ventilation rate for the model. *In vivo* tissue:blood partition coefficients were calculated by the area method of Gallo *et al.* (1987) from ia data obtained in the present study. The metabolic parameters, K_m and V_{max} , and blood:air coefficients were estimated from the observed data by nonlinear regression analysis (Table 1). Thus, the present model is a detailed and accurate description of the anatomy and physiology of the test subject, the male Sprague-Dawley rat. The inclusion of more individual tissues, as opposed to "lumped" tissue groups used in previous models, makes it possible to predict chemical concentrations in a larger number of specific target organs.

TABLE 1
Parameters Used in the Physiologically Based Pharmacokinetic Model for PCE in the Rat

Parameters	
Alveolar ventilation (ml/min)	132 ^a
Inhaled PCE concentration (mg/ml)	3.42 (509 ppm)
Body weight (g)	340
Alveolar mass transfer coefficient	500 ml/min
Tissue volumes	Percentages of body weight
Liver	3.39
Kidney	0.77
Fat	5.0
Heart	0.33
Lung	0.37
Muscle	35.36
Brain	0.6
Blood	7.4
Rest of body	46.78
Cardiac output	1.57 (ml/min · g) body wt (g) ^{0.75}
Blood flows	Percentages of cardiac output
Liver	15.73
Kidney	13.13
Fat	6.56
Heart	4.73
Lung	100
Muscle	26.11
Brain	2.21
Blood	100% = 1.57 (ml/min · g) body wt (g) ^{0.75}
Rest of body	31.53
Partition coefficients	
Blood:air	19.8
Fat:blood	152.5
Lung:blood	2.47
Liver:blood	5.25
Muscle:blood	2.98
Brain:blood	4.37
Heart:blood	2.68
Kidney:blood	4.45
Rest of body:blood	2.98
Metabolism constants	
<i>V</i> _{max} (μg/min)	0.15
<i>k</i> _m (μg/ml)	0.019

^a Alveolar ventilation for 50 ppm PCE exposure was determined to be slightly higher, at 151 ml/min, as experimentally determined by Dallas *et al.* (1994).

Areas under PCE concentration-time curves (AUCs) for blood and tissues were determined from the time of initiation of exposure to infinity. Total body clearance was determined by dividing the administered dose by the blood AUC. The maximum PCE concentration reached in blood and tissues (*C*_{max}) and the time after dosing that it occurred (*T*_{max}) were determined by observation of the available data points. The terminal elimination half-life (*t*_{1/2}) was determined according to the formula 0.693/β, where β is the terminal elimination rate constant.

Differential mass balance equations, incorporating the parameters listed in Table 1, were numerically integrated with the Advanced Continuous

Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, MA). The solution of the equations provided predicted PCE concentrations over time.

RESULTS

Measurement of PCE in tissues following its ia injection (Table 2, Figs. 2 and 3) revealed that the chemical was eliminated somewhat more rapidly from the liver than from other tissues. Extrahepatic tissues, other than fat and lung, exhibited similar *t*_{1/2} values, ranging from 412 to 443 min (Table 2). Elimination from the fat was relatively slow. The blood *t*_{1/2} was also quite long.

Tissue deposition, following ia injection of PCE, appeared to be governed by rate of blood perfusion and lipid content of the tissues. Highly perfused organs such as liver, kidney, and brain had relatively high *C*_{max} and AUC values. Nonlipoidal tissues, such as skeletal muscle, heart, and lung, had lower *C*_{max} and AUC values. The highest PCE levels were found in most tissues at the initial sampling time (i.e., 1 min post ia injection). The *T*_{max} for adipose tissue, however, was substantially longer. As would be anticipated for a chemical as highly lipophilic as PCE, the fat exhibited markedly higher *C*_{max} and AUC values than those of the other tissues.

Tissue:blood partition coefficients calculated from the ia tissue time-course data are included in Table 1. Due to the high degree of lipophilicity of PCE, the fat:blood value is almost two orders of magnitude greater than that for the nonfat tissues. Highly perfused tissues such as liver, kidney, and brain had similar values, which were approximately twofold larger than values for the other nonfat tissues.

Pharmacokinetic parameter estimates following the 2-hr inhalation exposure to 500 ppm PCE are presented in Table 3. The *C*_{max} achieved in adipose tissue was 9–18 times higher than in nonfat tissues. The AUC \int_0^∞ for fat was 45–80 times greater than AUC values for the nonfat tissues.

TABLE 2
Pharmacokinetic Parameter Estimates Following ia Injection of Rats with 10 mg PCE/kg body wt^a

Tissue	Area under curve \int_0^∞ (μg · min/ml)	Half-life (min)	<i>C</i> _{max} (μg/g)	<i>T</i> _{max} (min)
Liver	1737	389	41.0	1
Kidney	1759	412	36.0	1
Fat	60335	466	64.4	30
Heart	1059	439	19.0	1
Lung	909	479	11.3	1
Muscle	1178	443	5.7	10
Brain	1730	443	21.7	1
Blood	396	496	4.6	1

^a Each value represents the value for tissues of four rats pooled at each of 14 time-points, ranging from 1 min to 72 hr.

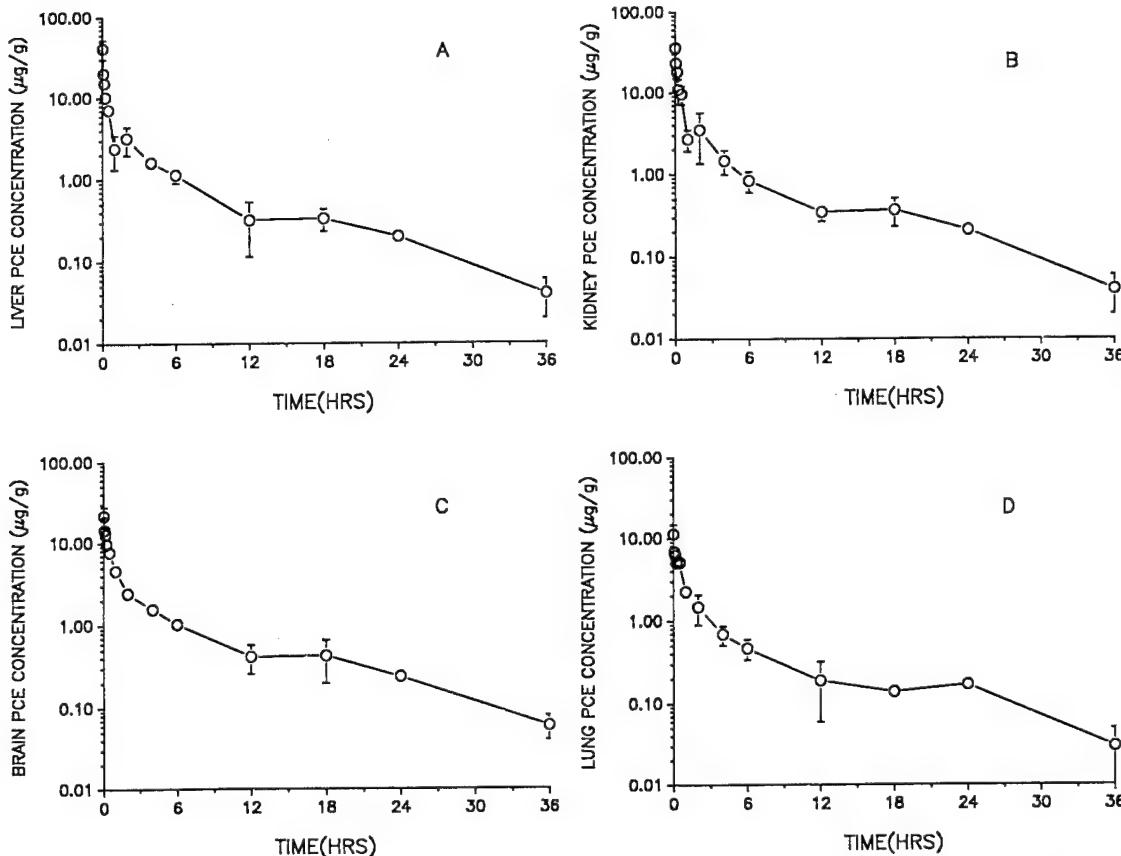


FIG. 2. PCE concentrations measured in the (A) liver, (B) kidney, (C) brain, and (D) lung of rats following ia injection of a single bolus of 10 mg/kg of PCE through an indwelling carotid arterial cannula. Each point represents the mean \pm SE for four rats.

Among nonfat tissues, the brain had the highest $AUC_{0\infty}$ and C_{max} values. Slightly lower values were observed for the liver. As anticipated for a moderately perfused, nonlipoidal tissue, skeletal muscle had the lowest C_{max} and shortest $t_{1/2}$. The muscle AUC was similar to that of tissue having a low lipid content (i.e., heart, kidney, and lung). Over the time course of inhalation exposures, the actual vapor concentration achieved in the chambers was 509 ± 23.7 ppm ($\bar{x} \pm SD$) ($n = 12$).

A PBPK model was developed, using the parameter estimates from the ia tissue disposition data and physiological values measured in male Sprague-Dawley rats, to predict the pharmacokinetics of PCE in the body during and following inhalation exposure. Model predictions of PCE tissue concentrations were compared to the experimentally determined (i.e., observed) PCE concentration versus time profiles in Figs. 4 and 5. Overall, concentrations of PCE both during and following inhalation exposure were well predicted by the model. Simulations of PCE levels in the liver and kidney were in close agreement with observed levels during and immediately following exposure, with very small overpredictions during the terminal elimination phase (Figs. 4A and 4C). Concentrations of PCE in the fat

were accurately forecast during the 2-hr inhalation period, while levels in heart, lung, brain, and blood were modestly but consistently underpredicted during this time. PCE elimination from extrahepatic tissues was quite accurately forecast during the 48- to 72-hr postexposure monitoring period. Fat concentrations were slightly underpredicted, and levels in each other tissue slightly overpredicted during the terminal elimination phase.

DISCUSSION

Findings in the present study demonstrate that PCE is very rapidly absorbed from the lungs into the systemic circulation and available for uptake by tissues throughout the body. Experiments in humans reveal that inhaled PCE resides in the alveolar air for only seconds before being absorbed (Opdam and Smolders, 1986). This is reflected in the current investigation by the observation of high blood PCE levels at the first sampling time (i.e., 1 min) after the beginning of exposures. Plots of PCE blood levels versus time of inhalation are atypical for halocarbons in that PCE levels continue to rise sharply throughout exposures. Fernandez *et al.* (1976) also reported a progressive increase in

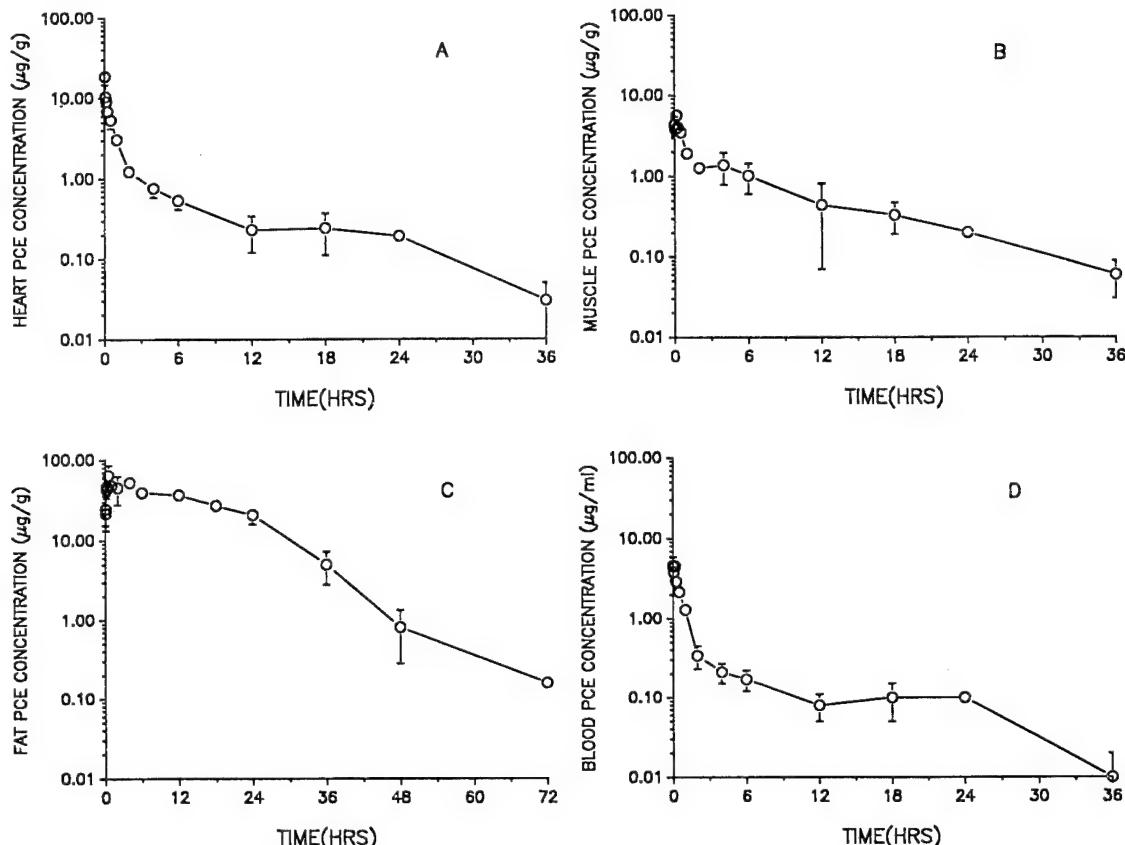


FIG. 3. PCE concentrations measured in the (A) heart, (B) skeletal muscle, (C) fat, and (D) blood of rats following ia injection of a single bolus of 10 mg/kg of PCE through an indwelling carotid arterial cannula. Each point represents the mean \pm SE for four rats.

PCE concentrations in the exhaled breath of persons during 8-hr exposures to 100 ppm PCE. Rapid approach to near steady-state concentrations is typically seen for other halocarbons, such as 1,1-dichloroethylene (Dallas *et al.*, 1983), TRI (Dallas *et al.*, 1989), and trichloroethylene (TCE) (Dallas *et al.*, 1991). These three halocarbons have short $t_{1/2}$ s, relative to PCE. As near steady state is generally reached in 4 to 5 $t_{1/2}$ s during ongoing exposures, the aforementioned profile for PCE would be anticipated, since the $t_{1/2}$ for PCE was determined here in ia experiments to be approximately 8 hr.

Results of the current study revealed that PCE is quickly taken up by most tissues upon inhalation or ia injection. PCE is a small, uncharged, lipid-soluble molecule which should rapidly diffuse through membranes of capillaries and other cells. This appears to be the case, since the T_{max} for most tissues following ia injection of PCE was 1 min (i.e., the initial sampling time). The T_{max} for skeletal muscle was somewhat longer (i.e., 10 min), likely the result of the slower perfusion rate and higher tissue mass of muscle relative to the other tissues. Adipose tissue, with the slowest perfusion rate, exhibited the longest T_{max} .

There is a paucity of data on the tissue distribution of PCE. Pegg *et al.* (1979) did publish blood PCE concentration-time profiles for postinhalation and for an oral exposure of rats. Pegg *et al.* (1979) and Frantz and Watanabe (1983) measured levels of radioactivity in several organs of rats at a single time point (i.e., 72 hr) post [^{14}C]PCE expo-

TABLE 3
Pharmacokinetic Parameters Estimates for a 2-hr Inhalation Exposure of Rats to 500 ppm PCE^a

Tissue	Area under curve \int_0^∞ ($\mu\text{g} \cdot \text{min}/\text{ml}$)	Half-life (min)	C_{max} ($\mu\text{g}/\text{g}$)
Liver	31247	423	152.4
Kidney	25868	425	107.5
Fat	1493190	578	1536.3
Heart	23179	328	106.6
Lung	18596	406	94.6
Muscle	24458	335	87.3
Brain	32975	455	173.9
Blood	8464	322	44.9

^a Each value represents the value for tissues of five rats pooled at each of 16 time points, ranging from 15 min after the initiation of exposure to 72 hr postexposure.

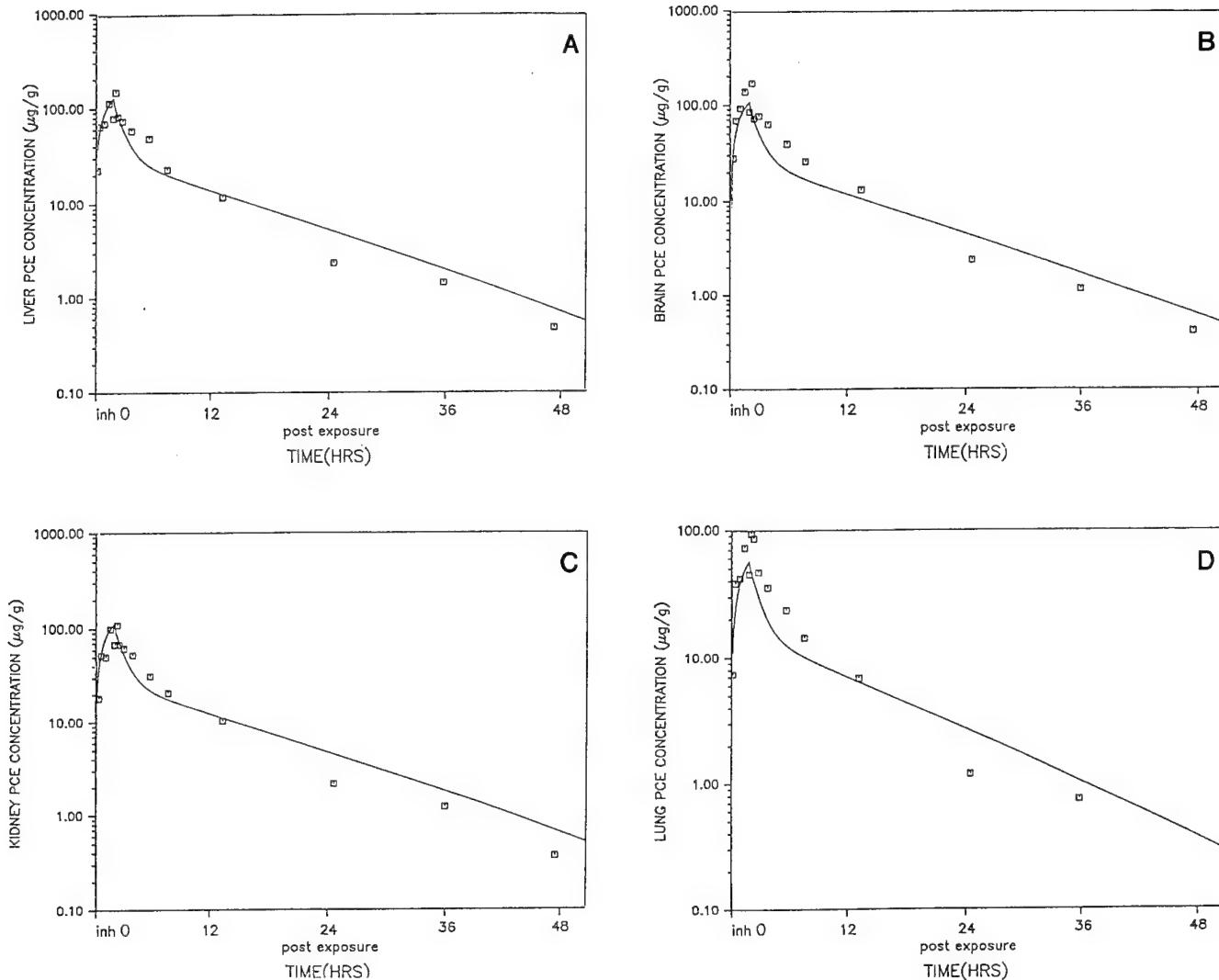


FIG. 4. Observed (□) and model-predicted (—) PCE concentrations in the (A) liver, (B) brain, (C) kidney, and (D) lung of rats during and following 2-hr inhalation exposures to 500 ppm PCE in rats. Each point represents the mean value for five rats.

sure. Pegg *et al.* (1979) did differentiate between total and irreversibly (i.e., covalently) bound radiolabel in the liver. Other investigators have examined autoradiograms of animals exposed to [¹⁴C]PCE vapors, in order to establish the qualitative distribution pattern of the chemical in the body. Ghatous *et al.* (1986) saw marked uptake of radioactivity in highly perfused tissues such as brain, liver, kidney, and lung, as well as in nasal mucosa, blood, and fat of mice after 10 min of inhalation of PCE. Savolainen *et al.* (1977) published the most extensive quantitative tissue data set to date. These researchers exposed male Sprague-Dawley rats 6 hr daily for 4 days to 200 ppm PCE, then euthanized two rats at each of the following times during the 5th day of exposure: 0, 2, 3, 4, and 6 hr. PCE levels were lowest in the blood and lungs, similar in the cerebrum and liver, and highest in perirenal fat. Marth (1987) found comparable PCE concentrations in the liver, brain, and kidney of mice

which consumed 0.05 or 0.1 mg/kg daily in their drinking water for 8 weeks. The spleen contained very high levels of PCE, which the investigator attributed to ongoing hemolysis and accumulation of PCE from the hemolyzed erythrocytes in the spleen. No comprehensive, scientifically sound tissue PCE concentration time-course profiles, however, were located for any route of exposure.

As mentioned under Results, the tissue distribution of PCE is apparently governed largely by the perfusion rate and the lipid content of organs. The liver, brain, and kidneys exhibited high PCE concentrations soon (i.e., 1 min) after i.v. injection of the chemical. All of the organs are highly perfused and each has a relatively high lipid content, except the kidneys. Two other lean, highly perfused organs, the heart and lungs, showed rapid PCE uptake, but low PCE concentrations. Skeletal muscle, a tissue with a moderate perfusion rate and low lipid content, exhibited relatively

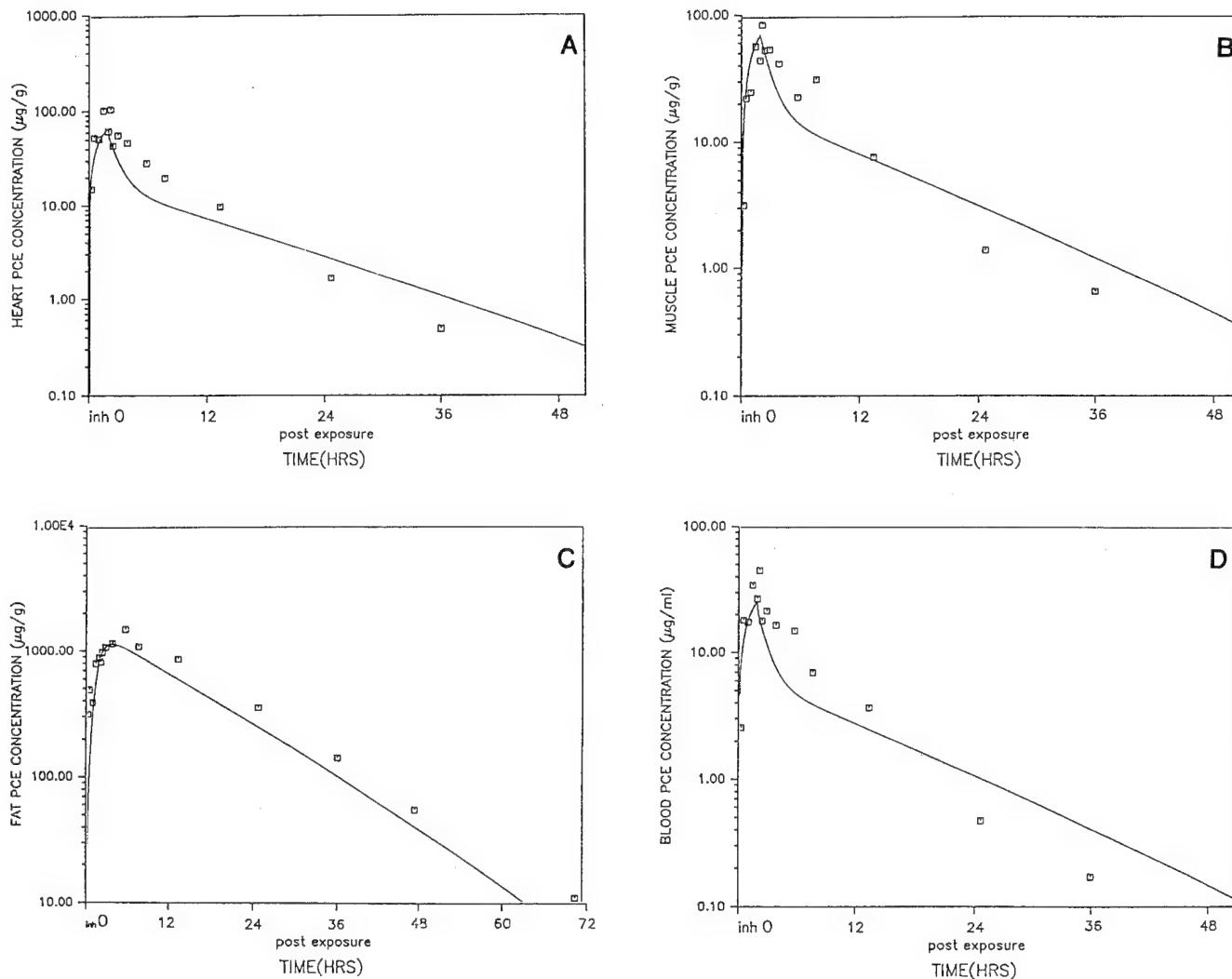


FIG. 5. Observed (□) and model-predicted (—) PCE concentrations in the (A) heart, (B) skeletal muscle, (C) fat, and (D) blood of rats during and following 2-hr inhalation exposures to 500 ppm PCE in rats. Each point represents the mean value for five rats.

slow, limited uptake of PCE. Bergman (1983) monitored the tissue distribution of a series of organic solvents in mice by whole-body autoradiography. He observed very rapid uptake of inhaled solvents such as TCE and carbon tetrachloride by the brain and other well-perfused organs. By 30 min postexposure, there had been redistribution of much of each halocarbon to adipose tissue. An analogous pattern was seen in the current experiments. PCE accumulated slowly but attained very high levels in fat. This phenomenon would be expected for deposition of a lipophilic chemical in a poorly perfused, lipoidal tissue. PCE levels progressively increased in each tissue, as they did in blood, during the 2-hr inhalation sessions. Savolainen *et al.* (1977) reported similar increases in rats throughout a 6-hr, 200 ppm PCE exposure.

PCE is eliminated from tissues of the body of the rat at slow but comparable rates. The $t_{1/2}$ s following ia injection

of a 10-mg/kg dose range from 389 min for liver to 496 min for blood (Table 2). The relatively rapid decline in PCE levels in the liver may reflect hepatic metabolism, although the capacity of rats to metabolize higher doses of PCE is limited (Pegg *et al.*, 1979; Schumann *et al.*, 1980). The blood $t_{1/2}$ of 8.27 hr in the current ia experiment is comparable to the values of 6.94–7.43 hr calculated by Pegg *et al.* (1979) and Frantz and Watanabe (1983) from inhalation and oral data. PCE which reenters the bloodstream from adipose tissue is, of course, available for reuptake by other tissues of the body. Thus, $t_{1/2}$ values for most organs monitored in the present study were quite similar. The slow systemic elimination of PCE may be attributed to a combination of factors including prolonged release from fat, limited metabolism, and a relatively high blood:air partition coefficient compared to those of other VOCs, resulting in prolonged exhalation.

In the present investigation, PBPK model-predicted PCE concentrations in tissues of rats were in close agreement with direct measurements of the chemical over time. Previous validations of PBPK models for PCE and other VOCs have had to primarily rely on observed blood and exhaled breath data (Chen and Blancato, 1987; Travis, 1987; Ward *et al.*, 1988). For example, the model of Reitz *et al.* (1988) reliably forecast blood and exhaled breath levels of TRI in mice, rats, and humans during and following inhalation exposures. Their PBPK model was versatile enough to forecast the kinetics of TRI in rats given the chemical iv and orally, as well as to predict target organ (i.e., liver) concentrations in humans who consume TRI in their drinking water. However, no actual tissue data were available for humans or other species for verification of the model predictions of target organ concentrations, other than levels of total radioactivity at a single time point (i.e., 6 hr) postexposure.

There have been relatively few attempts to model the uptake and elimination of halocarbons and other VOCs in tissues. In papers published to date (Ramsey and Andersen, 1984; Reitz *et al.*, 1988; Paustenbach *et al.*, 1988), the modelers had to utilize limited tissue concentration data of other investigators. Modeling was largely limited to simulations of VOC levels in the blood and fat. In the current investigation, tissue concentration versus time data from ia experiments were used to develop a PBPK model to describe the kinetics of PCE in blood and a variety of tissues and employed in simulating inhalation exposure. As described under Methods, the physiological and physicochemical input parameters were carefully measured in rats of the same sex and strain as those used in the PCE exposures. There have been considerable differences in the estimates of the metabolic parameters K_m and V_{max} for PBPK models of PCE in the rat (Hattis *et al.*, 1990), with differences between 60- and 15-fold, respectively, reported in the literature. Our V_{max} value is even lower than this range (2.9 nm/ml kg, in the units reported in that review article). However, these previous higher estimates of V_{max} were not based on es-liver concentration-time data, which were available for estimation of this parameter in the current study.

There were some dissimilarities between *in vivo* partition coefficients utilized in the current modeling effort and *in vitro* values used in many previous PBPK models. The *in vivo* blood:air partition coefficient that was determined for PCE was similar to an *in vitro* value (18.9) published by Gargas *et al.* (1989). The *in vivo* tissue:blood partition coefficients used in the present study, however, were 1.4, 1.8, and 2.8 times higher for liver, fat, and muscle, respectively, than *in vitro* values determined by Gargas *et al.* (1989). These differences in partition coefficients may be due to the use of Fischer-344 rats by Gargas *et al.* (1989) and Sprague-Dawley rats in the current investigation. The differences may also be attributable to the utilization of *in vitro* versus *in*

vivo techniques. The *in vitro* procedure of Gargas *et al.* (1989) involved the use of tissue homogenates, in which normal tissue architecture and cellular structure were disrupted. Artifactual changes may introduce changes in partitioning of PCE in some tissues. It seems preferable to utilize partition coefficients measured in animals during actual exposures, as these should be the most accurate parameters and therefore help reduce uncertainty in modeling. It was necessary for Ward *et al.* (1988), for example, to adjust the fat and muscle partition coefficients to obtain better fits to their experimental data. In the current modeling effort, predicted tissue concentration versus time data agreed quite well with PCE levels measured in tissues without having to alter any input parameter to optimize simulations. Thus the present model, used in conjunction with accurately determined *in vivo* input parameters, is a more detailed and representative description of physiological structure, which reliably predicts blood and tissue time courses of PCE in rats.

The use of experimental tissue concentration-time data and accurate measurement of physiological values can be of significant value in the development and validation of PBPK models. As such models can more reliably predict time integrals of target organ exposures to chemicals, they should improve the accuracy of risk assessments of PCE and other VOCs.

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APPENDIX D

Dallas, C.E., Muralidhara, S., Chen, X.M., Ramanathan, R., Varkonyi, P., Gallo, J.M., and Bruckner, J.V. "Use of physiologically-based model to predict systemic uptake and respiratory elimination of perchloroethylene." *Toxicology and Applied Pharmacology* 128: 60-68 (1994).

Use of a Physiologically Based Model to Predict Systemic Uptake and Respiratory Elimination of Perchloroethylene^{1,2}

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The pharmacokinetics of inhaled perchloroethylene (PCE) were studied in male Sprague-Dawley rats to characterize the pulmonary absorption and elimination of the volatile organic chemical (VOC). The direct measurements of the time course of PCE in the blood and breath were used to evaluate the ability of a physiologically based pharmacokinetic (PBPK) model to predict systemic uptake and elimination of PCE. Fifty or 500 ppm PCE was inhaled for 2 hr through a miniaturized one-way breathing valve by unanesthetized male Sprague-Dawley rats of 325–375 g. Serial samples of the inhaled and exhaled breath streams, as well as arterial blood, were collected during and following PCE inhalation and analyzed by headspace gas chromatography. PCE-exhaled breath concentrations increased rapidly to near steady state (i.e., within 20 min) and were directly proportional to the inhaled concentration. Uptake of PCE into the

blood was also rapid, but blood levels continued to increase progressively over the course of the 2-hr exposure at both exposure levels. Cumulative uptake, or total absorbed dose, was not proportional to the exposure level. A PBPK model was developed from *in vivo* parameters determined from tissue concentration-time data in a companion *ia* study (Dallas *et al.*, 1994, *Toxicol. Appl. Pharmacol.* 128, 50–59). PCE concentrations in the blood and exhaled breath during and following PCE inhalation were well predicted by the PBPK model. Despite species differences in blood:air and lung:air partition coefficients, the model was used to account for similar levels of PCE and other VOCs in the expired air of rats and humans. The model also accurately simulated percentage uptake and cumulative uptake of PCE over time. The model's ability to predict systemically absorbed doses of PCE under a variety of exposure scenarios should be useful in assessment of risks in occupational and environmental settings. © 1994 Academic Press, Inc.

Perchloroethylene (1,1,2,2-tetrachloroethylene) (PCE) is a volatile organic chemical (VOC) which is used in large quantities in industry for metal degreasing, dry cleaning of fabrics and textiles, and as an intermediate in the production of other chemicals. Approximately 500,000 workers in the United States are estimated to be occupationally exposed to PCE (NIOSH, 1978). Measurements of workplace air concentrations of PCE in the dry cleaning industry have yielded mean 8-hr time-weighted averages of 28.2–88.2 ppm (Materna, 1985) and 4–149 ppm (Ludwig *et al.*, 1983). PCE has been found in indoor air in residences using PER-contaminated water supplies (Highland *et al.*, 1985; Andelman, 1985). Central nervous system (CNS) effects including dizziness, headache, sleepiness, incoordination, and impairment of performance on psychophysiological tests have been reported in humans acutely exposed to levels of 100–200 ppm PCE and higher (Stewart *et al.*, 1961, 1970; Hake and Stewart, 1977). High-level inhalation exposures to PCE have also been reported to result in mild hepatorenal toxicity in some humans (Hake and Stewart, 1977), as well as mild hepatotoxicity (Kylin *et al.*, 1963), biochemical changes in the brain including reduced RNA content (Sa-

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volainen *et al.*, 1977), and CNS depressant effects (Rowe *et al.*, 1952; Goldberg *et al.*, 1964) in laboratory animals.

Although PCE produces CNS depression as well as mild hepatorenal toxicity in sufficiently high doses, its potential as a carcinogen is of primary concern at low occupational and environmental levels. High, chronic doses of PCE have been shown to cause an increased incidence in hepatocellular carcinomas in B6C3F1 mice but not in rats (NCI, 1977; NTP, 1986). There was also an increase in mononuclear cell leukemia in male and female Fischer 344 rats, as well as a low incidence of renal tubular cell tumors in the male rats (NTP, 1986). Some epidemiological studies of dry cleaning workers have not found an excess incidence of cancer (Brown and Kaplan, 1987), while others have (Duh and Asal, 1984). Such investigations have stimulated interest in conducting valid cancer risk assessments of PCE (ATSDR, 1993).

Physiologically based pharmacokinetic (PBPK) models have been used increasingly in risk assessments of PCE and other VOCs, particularly in the species-to-species and high-to-low dose extrapolations often necessary to apply animal bioassay data to low-level human exposures. It is important to determine the quantity of chemical absorbed systemically (i.e., the absorbed dose), as it is often quite different from the inhalation or oral exposure level (i.e., the administered dose). Systemic absorption of inhaled PCE has been demonstrated to be dependent upon a number of factors, including lean body mass, respiratory rate, duration of exposure, and inhaled concentration in limited human experiments (Hake and Stewart, 1977; Monster, 1979). Valid PBPK models offer one the ability to predict systemic uptake and elimination of chemicals in laboratory animals and humans under a variety of exposure conditions. A PBPK model for PCE was recently developed and validated in a companion paper (Dallas *et al.*, 1994). The model was based on a time-course data sets from laboratory experiments in rats, and the accuracy of its predictions verified by comparison with the actual time course of PCE in tissues of rats inhaling the chemical. The current work was undertaken to determine whether this model can accurately forecast systemic uptake of PCE over time as well as respiratory elimination of the volatile halocarbon.

Objectives of this study were to (1) directly determine the systemically absorbed dose of PCE during inhalation exposures, by simultaneously measuring PCE in the inhaled and exhaled breath; and (2) evaluate the ability of a recently developed PBPK model (Dallas *et al.*, 1994) to accurately predict the systemic uptake and elimination of PCE, by comparison of simulated and observed levels of the chemical over time in the blood and breath of rats.

METHODS

Animals. Adult, male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr

and dark from 1900 to 0700 hr. They were housed in stainless steel cages in a ventilated animal rack. Tap water and Ralston Purina Formula Chow were provided *ad libitum*. The rats were used after at least a 14-day acclimation period, at which time their body weight ranged from 325 to 375 g. Solvent exposures were initiated at approximately the same time each day (1000–1200 hr).

Test material. Perchloroethylene (tetrachloroethylene), of 99%+ purity, was obtained from Aldrich Chemical Company (Milwaukee, WI). The purity of the chemical was verified by gas chromatographic analysis.

Animal preparation. An indwelling carotid artery cannula was surgically implanted into each animal. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml), acepromazine maleate (10 mg/ml), and xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v/v/v). The cannulated animals were freely moving during a 24-hr recovery period.

Inhalation exposures. A face mask specifically designed to fit the Sprague-Dawley rat of this size was held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. A miniaturized one-way breathing valve (Hans Rudolph, Inc., St. Louis, MO) was attached to the face mask so that the valve entry port was directly adjacent to the nares of the animal. This established separate and distinct airways for the inhaled- and exhaled-breath streams. The use of such a device for pharmacokinetic studies of inhaled halocarbons in small animals has been described in detail (Dallas *et al.*, 1986). A known concentration of PCE was generated within a 70-liter gas sampling bag (Calibrated Instruments, Ardsley, NY) by injecting the appropriate quantity of the VOC into the bag filled with air. Uniform dispersion of the vapor was insured by a magnetic stirring bar within the bag. The bag was then connected in series by Teflon tubing with a pneumotachograph, the one-way breathing valve, and an empty 70-liter gas collection bag. Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. Each cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposures (Battelle-Geneve, Switzerland). PCE inhalation exposures of 2-hr duration were initiated only after stable breathing patterns (i.e., respiratory rate and minute volume) were established. During this exposure period and for up to 8 hr afterward, serial-inhaled and exhaled-breath samples were taken at approximately the same time as blood samples from the carotid artery cannula. Both air and blood samples were then analyzed for PCE content by headspace gas chromatography.

Respiratory measurements and calculations. In order to calculate the dose of PCE absorbed systemically during inhalation exposures, the respiration of each animal was continuously monitored. The respiratory monitoring was conducted according to methods previously published in solvent exposure studies by this laboratory (Dallas *et al.*, 1983, 1986, and 1989). The airflow created by each animal's inspiration was recorded both during and following PCE exposure in terms of minute volume (V_E), respiratory rate (f), and tidal volume (V_T). An average value for these parameters for each animal was obtained from measurements taken at 15-min intervals during the 2-hr exposures. The mean \pm SD of these values for the 500-ppm exposure group ($n = 6$) were $V_E = 189 \pm 21.5$ ml/min, $f = 119.1 \pm 22.4$ breaths/min, $V_T = 1.62 \pm 0.34$ ml. The mean \pm SD for the corresponding values for the 50-ppm exposure group ($n = 6$) were $V_E = 216 \pm 43.1$, $f = 134.5 \pm 14.9$, $V_T = 1.67 \pm 0.36$.

Calculations of PCE uptake and elimination were conducted utilizing equations presented in a previous VOC inhalation study in rats (Dallas *et al.*, 1989). Since the V_E and the exhaled breath PCE concentration at each sampling point were measured, subtraction of the quantity of PCE exhaled from the amount inhaled yielded the quantity of PCE taken up during sequential sampling periods. By summing these values, the cumulative uptake, or systemically absorbed dose, was determined. The percentage uptake during each exposure period was determined by dividing the cumulative uptake by the total inhaled dose for that time period.

A PBPK model was used to describe the uptake and elimination of PCE in the rat. It was assumed that a blood-flow-limited model was appropriate

to characterize the tissue distribution of PCE. Most previous PBPK models for VOCs have utilized blood-flow-limited organ representations (Ramsey and Andersen, 1984; Angelo and Pritchard, 1984; Ward *et al.*, 1988; Dallas *et al.*, 1989, 1991). Compartmental volumes and organ blood flows were determined in this laboratory for the male Sprague-Dawley rat (Delp *et al.*, 1991; Manning *et al.*, 1991). *In vivo* tissue:blood partition coefficients were calculated from tissue concentration-time data for PCE following its i.v. injection in a companion study (Dallas *et al.*, 1993) using the area method of Gallo *et al.* (1987). The metabolic parameters K_m and V_{max} and blood:air partition coefficients were estimated from the observed i.v. time-course data for liver and blood by nonlinear regression analysis. Alveolar ventilation was determined to be 50% of the minute volume, accounting for dead space in the animal and in the miniaturized breathing valve. Alveolar ventilation values were measured in the present study. The alveolar mass transfer coefficient was based on the alveolar permeability-area product for methylene chloride (Angelo and Pritchard, 1984). The lung:air partition coefficient was derived using the area method by Gallo *et al.* (1987). Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport of PCE in the rat were numerically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, MA). The solution of the equations provided predicted PCE blood, expired air, and tissue concentrations over time. The model-predicted cumulative uptake values were the sum of the simulated amounts of PCE in each tissue compartment in the model.

Analysis of PCE in air and blood. The concentrations of PCE in the inhaled- and exhaled-breath samples collected during inhalation exposures were measured with a Tracor MT560 gas chromatograph (GC) (Tracor Instruments, Austin, TX). Standards were prepared in each of four 9-liter bottles equipped with Teflon stoppers with needles from which air samples could be taken with a syringe. Analyses for the 500 ppm exposures were conducted using a flame ionization detector (FID), while the analyses for the 50-ppm exposures were conducted using an electron capture detector (ECD). In either case, air samples were procured with a gas-tight, 1-ml syringe and injected directly onto an 8-ft \times $\frac{1}{8}$ -in stainless steel column packed with 0.1% AT 1000 on GraphPak. Operating temperatures were 150°C, injection port; 200°C, FID detector; 350°C, ECD detector; and 110°C, isothermal column operation. When using the ECD, gas flow rates of 40 ml/min were employed for nitrogen (carrier gas) with an additional make-up gas flow rate to the detector of 20 ml/min.

Concentrations of PCE in the blood were measured by a GC headspace technique (Chen *et al.*, 1993). Blood samples were withdrawn from the arterial cannula via a stopcock into a 1-ml syringe. Depending on the anticipated blood PCE concentration, from 25 to 200 μ l of the blood was taken from the stopcock with an Eppendorf pipette and transferred to chilled headspace vials (Perkin-Elmer, Norwalk, CT). These vials were capped immediately with PTFE-lined butyl rubber septa and metal washers and tightly crimped. Each sample vial was then placed into the HS-6 autosampler unit of a Sigma 300 GC (Perkin-Elmer) where it was heated to 90°C by a thermostat. A predetermined volume of the vapor was then injected automatically into the column for analysis. The column used was an 8-ft \times $\frac{1}{8}$ -in stainless steel column packed with 10% FFAP chroma-sorb W-AW (80–100 mesh). Operating temperatures were 200°C, injection port; 400°C, ECD detector; and 110°C column oven. The carrier gas was 5% argon-methane at a flow rate of 40 ml/min with a make-up gas flow rate of 20 ml/min to the detector. The limit of detection was about 1 ng, or 6.7 ppb, in air. The percentage recovery from spiked blood samples was approximately 95% (Chen *et al.*, 1993).

RESULTS

PCE concentrations inhaled by the animals were determined by analysis of air samples taken from a sampling port immediately adjacent to the breathing valve. Actual inhaled PCE concentrations for the six rats in each group

TABLE 1
Parameters Used in the Physiologically Based Pharmacokinetic Model for PCE in the Rat

Parameters	
Alveolar ventilation ml/min	108 (50 ppm); 94.5 (500 ppm)
Inhaled PCE concentration (mg/ml)	0.35 (50 ppm); 3.55 (500 ppm)
Body weight (g)	340
Alveolar mass transfer coefficient	500 ml/min
Tissue volumes (ml)	Percentages of body weight
Liver	3.39
Kidney	0.77
Fat	5.0
Heart	0.33
Lung	0.37
Muscle	35.36
Brain	0.6
Blood	7.40
Rest of body	46.78
Cardiac output	1.57 (ml/min · g) body wt (g) ^{0.75}
Blood flows (ml/min)	Percentages of cardiac output
Liver	15.73
Kidney	13.13
Fat	6.56
Heart	4.73
Lung	100
Muscle	26.11
Brain	2.21
Blood	100% = 1.57 (ml/min · g) body wt (g) ^{0.75}
Rest of body	31.53
Partition coefficients	
Blood:air	18.9
Fat:blood	152.5
Lung:blood	2.48
Liver:blood	5.25
Muscle:blood	2.98
Brain:blood	4.37
Heart:blood	2.68
Kidney:blood	4.45
Rest of body:blood	2.98
Metabolism constants	
V_{max} (μ g/min)	0.15
K_m (μ g/ml)	0.019

were 528.2 ± 21.9 ppm ($\bar{x} \pm SD$) for the 500-ppm exposures and 53.1 ± 5.1 ppm ($\bar{x} \pm SD$) for the 50-ppm exposures.

Substantial respiratory elimination of unchanged PCE was evident during the 2-hr inhalation exposure period. Near steady-state PCE levels were achieved in the exhaled breath within 20 min and maintained for the duration of the exposures. The near steady-state concentrations were 2.1–2.4 μ g/ml in the exhaled air of the 500 ppm rats (Fig. 1B) and 0.20–0.22 μ g/ml in the 50 ppm animals (Fig. 2B). Thus, the exhaled breath levels during the 2-hr exposures

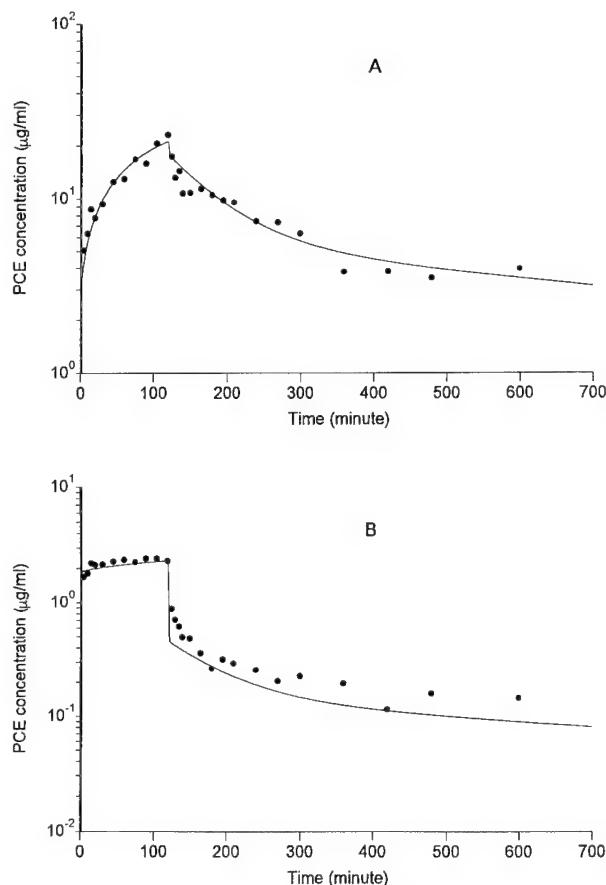


FIG. 1. Observed (●) and predicted (—) PCE concentrations in the (A) arterial blood and (B) exhaled air of rats during and following a 2-hr, 500 ppm inhalation exposure. Each point represents the mean value for six rats.

were directly proportional to the inhaled PCE concentrations.

PCE was rapidly absorbed from the lungs, as relatively high arterial blood concentrations of PCE were measured at the first sampling time (i.e., 2 min). Unlike the pattern of PCE levels in the exhaled breath, the concentration of PCE in the blood progressively increased over the course of the 2-hr session at both exposure levels. The rates of increase of the PCE concentrations in the blood were greater in the 500 ppm group (Fig. 1A) than those in the 50 ppm group (Fig. 2A). Arterial blood PCE concentrations were not proportional to the inhaled concentrations. After the initial rapid uptake phase during the first 30 to 60 min of exposure, blood levels in the 500 ppm rats were 12 to 17 times higher than those in the 50 ppm rats. Upon cessation of PCE inhalation, the chemical was eliminated very quickly during the first minutes, particularly from the exhaled breath. Following this initial phase, PCE levels in the blood and breath declined slowly.

Plots of the cumulative uptake of PCE over time are shown in Fig. 3. Visual observation of the measured data

points reveals a more pronounced deviation from linearity in the 500 ppm group than that in the 50 ppm group. Total cumulative uptake of PCE during the 2-hr exposure to 500 ppm was determined to be 28.1 ± 4.3 mg ($\bar{x} \pm SD$), or 79.9 mg/kg body wt. The 2-hr exposure to 50 ppm PCE resulted in a cumulative uptake of 3.9 ± 0.9 mg ($\bar{x} \pm SD$), or 11.2 mg/kg body wt. Thus, cumulative uptake was not proportional to inhaled concentration. The percentage systemic uptake of PCE is shown in Fig. 4. Percentage uptake was relatively constant after the first 20 min of inhalation, approximately 40% in the 500 ppm group and approximately 50% in the 50 ppm group.

The PBPK model predictions of blood and exhaled-breath concentrations of PCE are shown in Figs. 1 and 2. Both observed and predicted exhaled-breath concentrations of PCE rapidly achieved near steady state following the initiation of exposures. Predicted concentrations of PCE in the exhaled air both during and following 500 ppm (Fig. 1B) and 50 ppm (Fig. 2B) inhalation exposures agreed very well with the observed concentrations. The steep increase in PCE blood concentrations throughout the 500

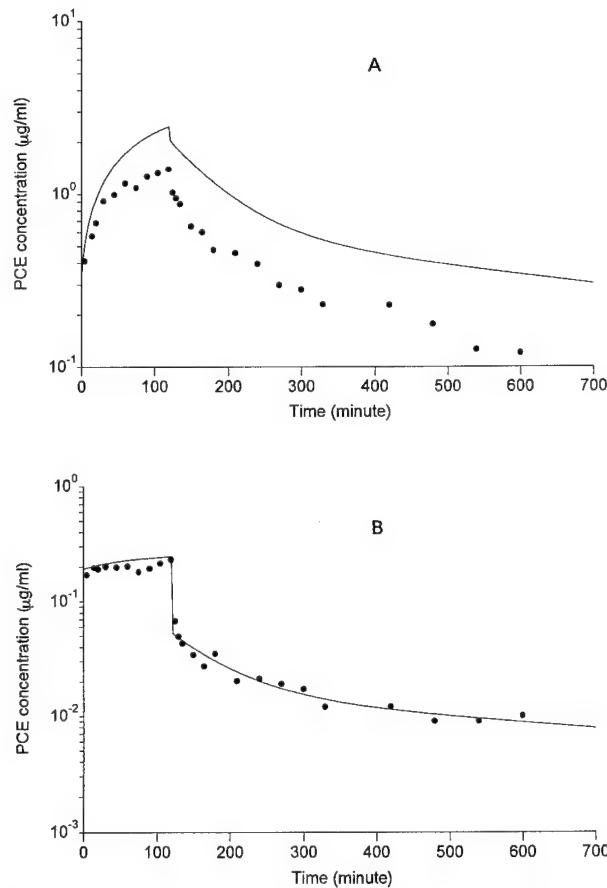


FIG. 2. Observed (●) and predicted (—) PCE concentrations in the (A) arterial blood and (B) exhaled air during and following a 2-hr, 50 ppm inhalation exposure. Each point represents the mean value for six rats.

ppm exposure (Fig. 1A) was accurately simulated by the model. PCE blood concentrations postexposure were also well simulated. The uptake of PCE in the blood during the second hour of the 50 ppm exposure (Fig. 2A) was overpredicted, as were postexposure blood levels. A saturable, high-affinity metabolic pathway with a somewhat greater capacity could have accounted for this disparity in predicted and measured blood levels. We adopted the practice, however, of using a training data set from a separate experiment (i.e., V_{max} and K_m based on the ia data in the companion study (Dallas *et al.*, 1993)) to derive input parameters for the PBPK model to obtain predictions in the current study.

The PBPK model was also utilized to generate predictions of cumulative uptake and percentage uptake of PCE during the 2-hr inhalation sessions. Cumulative uptake of PCE was well predicted over the course of the 500 ppm inhalation exposure (Fig. 3A). During the 50-ppm exposure, a slight underprediction during the initial hour of PCE inhalation became more pronounced during the last hour, resulting in a forecast of cumulative uptake which was 20% less than the observed value at 120 min (Fig. 3B). In the second hour of the 500 ppm exposure, model simulations

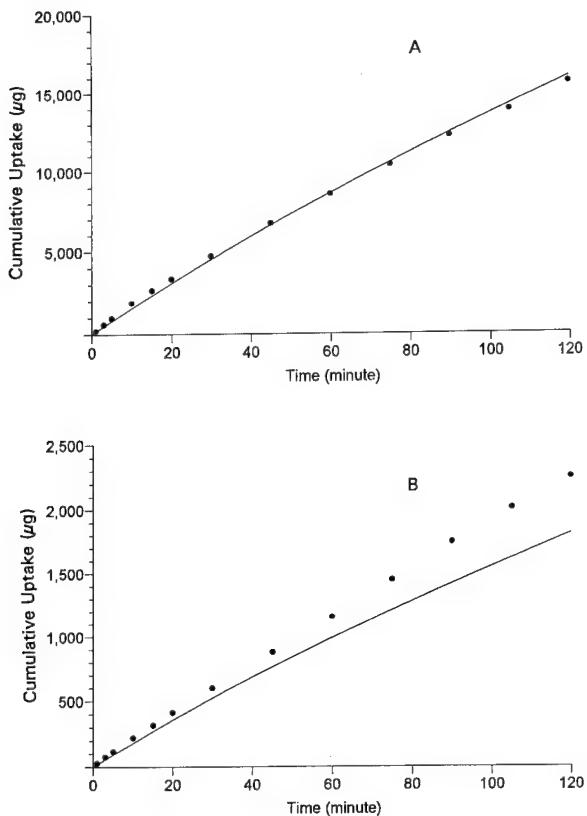


FIG. 3. Cumulative uptake of PCE during inhalation of 500 (A) or 50 (B) ppm PCE for 2 hr. The quantity of PCE retained during successive 10-min intervals was calculated as described under Methods. Each point represents the mean for six rats. Model predictions of cumulative uptake are delineated by the solid lines.

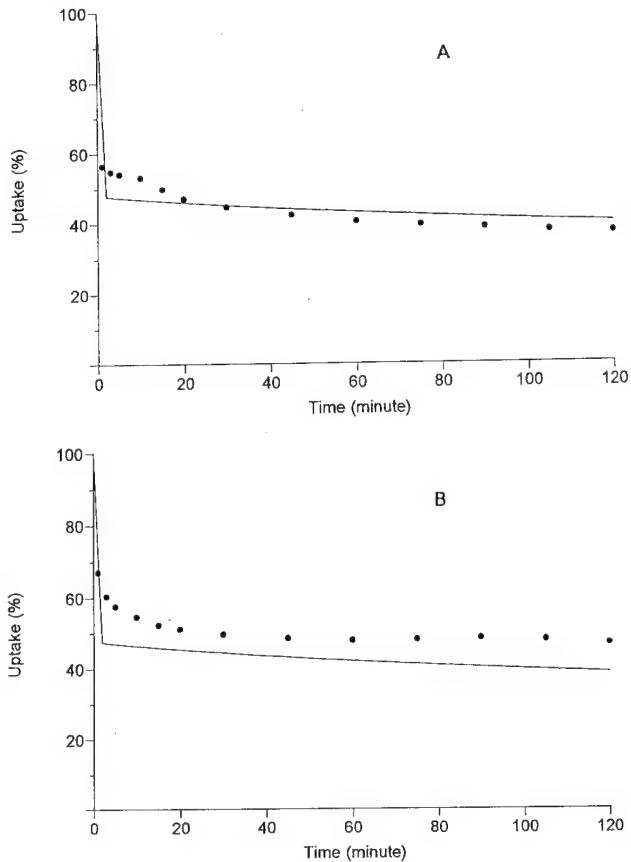


FIG. 4. The percentage systemic uptake of PCE over time during inhalation exposures to 500 (A) or 50 (B) ppm PCE for 2 hr. Each point represents the mean for six rats. The percentage uptake of the inhaled dose was determined after 1, 3, 5, 10, 15, and 20 min and at 10-min intervals thereafter, as described under Methods. Model predictions of the percentage uptake are delineated by the solid lines.

of percentage uptake were within 2% of the observed values (Fig. 4A). For the 50 ppm exposure, the predicted percentage uptake was consistently about 5% below the observed percentage uptake (Fig. 4B). As discussed earlier, a somewhat greater metabolic rate in the rats used in the current investigation than in those used in the companion ia study by Dallas *et al.* (1993) could be responsible for the underprediction of percentage systemic uptake.

Model predictions of exhaled breath concentrations were also conducted using different lung:air partition coefficients in order to determine the influence of this parameter on exhalation of PCE in dissimilar species. Employing the assumption of equivalent tissue:blood coefficients in species, including lung:blood partition coefficients, a lung:air partition coefficient was calculated by use of the equation

$$r_a = r_l r_{b:a},$$

where r_a is lung:air partition coefficient, r_l is lung:blood partition coefficient, and $r_{b:a}$ is blood:air partition coefficient.

TABLE 2
Predicted PCE Exhaled Breath Concentrations [$\mu\text{g}/\text{ml}$]
Using Different r_a Values^a

r_a	50 ppm		500 ppm	
	1 hr	2 hr	1 hr	2 hr
46.87 (rat)	0.25	0.26	2.36	2.48
25.54 (human)	0.26	0.28	2.40	2.70

^a The influence of different r_a (lung:air partition coefficient) values on exhaled breath concentrations predicted by a PBPK model is evaluated for a 1- or 2-hr inhalation exposure to 50 or 500 ppm PCE in rats and humans.

In the current rat model, $r_a = 46.87$, $r_l = 2.48$, and $r_{ba} = 18.9$. Use of the human r_{ba} value of 10.3 of Bois *et al.* (1990) and the r_l of 2.48 resulted in a human r_a of 25.54. Model predictions of PCE exhalation over time were made using these two r_a values of 46.87 for rats and 25.54 for humans. The predictions of exhaled PCE concentrations in rats and humans after 1 and 2 hr of exposure are included in Table 2. The entire time courses for the 500 and 50 ppm exposures are shown in Figs. 5A and 5B, respectively. It can be seen that even a doubling of the lung:air partition coefficient resulted in only a small difference in exhaled concentrations in rats and humans at each exposure level. The larger r_a value resulted in a slightly lower predicted PCE concentration in exhaled breath.

DISCUSSION

PCE is readily absorbed from the lungs into the blood. This is manifest in rats by a substantial percentage uptake and relatively high arterial blood levels at the initial sampling times (i.e., 1 and 2 min, respectively) after inhalation of PCE begins (Figs. 1A and 2A). Although percentage uptake is initially quite high, it decreases as a function of time for the first 20 min. Systemic uptake remains relatively constant thereafter for the duration of the 2-hr exposure (Fig. 4), with percentage uptake slightly higher (i.e., 50%) at the lower (i.e., 50 ppm) exposure level. This finding is evidence of a limited capacity of the male Sprague-Dawley rat to metabolize PCE. The plots of cumulative uptake, as well as total uptake provide evidence of saturable PCE metabolism in Sprague-Dawley rats. Deviation from linearity in plots of cumulative uptake versus time is more evident at the higher (i.e., 500 ppm) exposure level. The 10-fold increase in administered dose results in just a 7.2-fold increase in the total absorbed dose over the 2-hr exposure. This phenomenon of saturable metabolism of PCE in Sprague-Dawley rats is also reported by Schumann *et al.* (1980) and Pegg *et al.* (1979). The latter group report that B6C3F1 mice have a significantly greater capacity to metabolize PCE than do the rats. Humans appear to have a quite limited capacity to

metabolize PCE. The data of Ohtsuki *et al.* (1983) and Seiji *et al.* (1989) demonstrate that PCE metabolism is saturated at inhaled concentrations >100 ppm. Uptake of inhaled concentrations of 72–144 ppm PCE diminishes as a function of time in humans, decreasing to approximately 62% (Monster *et al.*, 1979), a value somewhat higher than those measured in rats in the current study.

The systemic uptake and elimination patterns of PCE are characteristic of a lipid-soluble, poorly metabolized chemical. Uptake of an inhaled halocarbon should be determined largely by its solubility in the blood and tissues and its rate of metabolism (Monster, 1979). Blood levels progressively increased during the 2-hr exposures in the current study, though they seemed to be approaching steady state by 2 hr in the 50 ppm group. Stewart *et al.* (1961) saw a similar pattern in blood levels in humans inhaling 194 ppm PCE, with apparent steady state reached after 3 hr of exposure. PCE concentrations in tissues, as in blood, steadily increased in rats inhaling 500 ppm of the halocarbon (Dallas

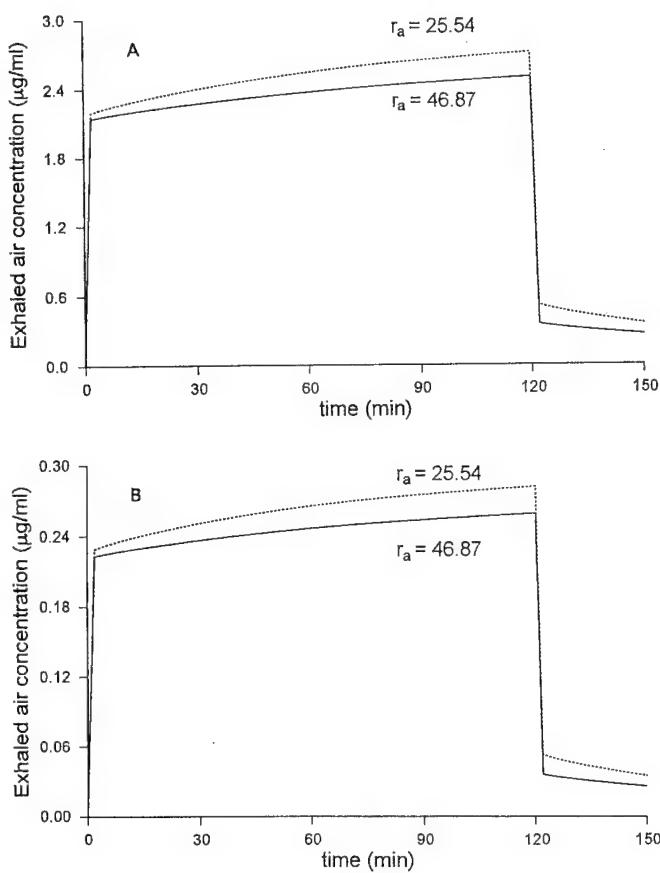


FIG. 5. Model predictions of exhaled breath PCE concentrations using different lung:air partition coefficients (r_a). Lung:air partition coefficients of 46.87 for rats and 25.54 for humans were calculated according to the equation described under Results. Predicted levels of PCE in the exhaled breath of humans (dashed lines) and rats (solid lines) are compared during and following 500 (A) and 50 (B) ppm exposures to PCE.

et al., 1993). The pattern of uptake into and elimination from fat was quite different. PCE uptake by fat was slower but much greater in magnitude, due to a low perfusion rate and a very high adipose tissue:blood partition coefficient. Although blood samples were not taken long enough postexposure in this companion study to define the terminal elimination phase, blood levels appeared to diminish more rapidly in the low-dose (i.e., 50 ppm) animals. This is indicative that metabolism, albeit of limited capacity, contributes to the systemic clearance of lower doses of PCE.

As for many other VOCs, the major route of elimination of PCE in laboratory animals and man is exhalation of the parent compound. There have been several studies involving direct measurements of the respiratory elimination of PCE in humans. This allows interspecies comparisons of the quantity of the halocarbon which is eliminated in the breath. Following inhalation of 100 ppm by humans for 2 hr, PCE concentrations in the exhaled breath at 1 and 2 hr postexposure were 0.06 and 0.047 $\mu\text{g}/\text{ml}$. Assuming a linear scale-up from the 50 ppm data in the current investigation to 100 ppm, the PCE concentration in the expired air of rats at these two time points would be 0.07 and 0.04 $\mu\text{g}/\text{ml}$, respectively. Similarly, postexposure exhaled breath PCE levels in other human studies (Stewart *et al.*, 1961, 1970) were comparable to levels of PCE measured in the breath of rats in the current study. Similar concentrations of halocarbons in the expired air of rats and humans were also noted in studies of 1,1,1-trichloroethane (TRI) (Dallas *et al.*, 1989) and 1,1,2-trichloroethylene (TCE) (Dallas *et al.*, 1991). Since the blood:air partition coefficients for PCE, TRI, and TCE are markedly higher in rats than in humans (Gargas *et al.*, 1989), it might be anticipated that this physicochemical difference would result in greater respiratory elimination of the halocarbons by humans when there are equivalent blood levels.

Consideration of species differences in blood:air and tissue:air partition coefficients is necessary in order to understand why human and rat exhaled-breath concentrations can be so similar. In the PBPK model of Bois *et al.* (1990) for PCE, rat blood:air and tissue:air partition coefficients are significantly greater than corresponding human partition coefficients. When one uses these values to calculate tissue:blood coefficients, the tissue:blood coefficients for rats and humans are quite similar.

In the current PBPK model, chemical input occurs at the lung:air interface and is characterized by a mass transfer coefficient and a lung:air partition coefficient. Since the value of the mass transfer coefficient would not be rate-limiting for chemical uptake, the influence of changes in the lung:air partition coefficient (r_a) on exhaled breath concentration was assessed. Model simulations were conducted with two different r_a values, calculated as described under Results, for rats and for humans. The only model parameter that varied in the comparison was r_a . The differences

between the exhaled PCE concentrations predicted using the two different r_a values were very small, for both the 50 and the 500 ppm exposures. It can be anticipated, then, that the concentration of PCE exhaled by humans and rats can be similar, despite dissimilar blood:air, or lung:air partition coefficients. This disparity in blood:air partition coefficients may be offset by higher tissue:pulmonary perfusion and respiratory rates in rats.

Overall, concentrations of PCE in the blood and breath were accurately predicted during and following inhalation exposures by the current PBPK model. Only in the 50 ppm rats were arterial blood levels overpredicted. The reason for this discrepancy is unclear. There have been few other attempts to model the time course of PCE in blood or exhaled breath. The percentage of PCE exhaled over time following inhalation of the halocarbon was well predicted in rats by a PBPK model of Ward *et al.* (1988). These investigators compared their simulated values to experimental data previously published by Pegg *et al.* (1979). It was necessary for Ward and co-workers, however, to increase their fat:air partition coefficient from 1638 to 2300 in order to more closely simulate PCE concentrations in expired air. The *in vivo* fat:blood partition coefficient of 152.5, derived in our companion study (Dallas *et al.*, 1993) from adipose tissue time-course data, corresponds to a fat:air partition coefficient of 2881. Guberan and Fernandez (1974) developed a PBPK model with four compartments, which they used to make preliminary forecasts of alveolar PCE concentrations in humans who inhaled the chemical. In subsequent work, Ward *et al.* (1988) utilized their own PBPK model and the experimental human data of Stewart *et al.* (1961) and Fernandez *et al.* (1976). Predictions by the model of Ward and his colleagues of PCE levels in expired air during and post inhalation exposures were in close agreement with the empirical data. No one to our knowledge, however, has previously modeled the time course of PCE in the bloodstream during or following inhalation exposures, until the current series of experiments described herein and in the companion paper (Dallas *et al.*, 1994).

The ability of the present PBPK model to predict the disposition of inhaled PCE should make it useful in health risk assessments in occupational and environmental settings. One of the model's most important assets is its ability to accurately forecast systemic uptake of the chemical over time over a range of exposure concentrations. Although risk assessments often have been based upon the administered dose, it is now accepted that the (systemically) absorbed dose should be determined and utilized instead. The absorbed dose may be time-, concentration-, and species-dependent. Although Fernandez *et al.* (1976) measured alveolar PCE levels in humans and noted that uptake is the product of the minute volume and the difference between inspired and alveolar concentrations, no reports of systemic absorption of PCE by their or other groups of investigators

were found in the literature. Time integrals of target organ exposure to PCE, as described in the companion study (Dallas *et al.*, 1993), can be quite useful in conducting risk assessments. Andersen (1987) points out that the most appropriate tissue dose surrogate is a time integral of the concentration of active form of a chemical. The CNS depressant and cardiac arrhythmogenic actions of PCE are caused by the parent compound. It is generally accepted that one or more reactive metabolites are responsible for PCE's cytotoxic (Buben and O'Flaherty (1985) and carcinogenic actions (ATSDR, 1993), but it is not clear which metabolite(s) are responsible. Thus, a number of researchers have used PBPK models to predict total PCE metabolism and associated cancer risks (Chen and Blancato, 1987; Bogen and McKone, 1988; Bois *et al.*, 1990). Hattis *et al.* (1990) examined the appreciable differences in predictions of risk among different PCE models and found they were largely due to the choice of disparate metabolic parameters. There were also disagreements about the relative importance of competing linear and saturable metabolic pathways in different species. Thus, it is clear that more research should be conducted on the mechanisms of PCE cytotoxicity and carcinogenicity, so the appropriate active metabolite(s) can be identified and monitored in future experiments and PBPK modeling efforts.

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APPENDIX E

Dallas, C.E., Chen, X.M., Muralidhara, S., Varkonyi, P., Tackett, R.L., and Bruckner, J.V.
"Use of tissue disposition data from rats and dogs to determine species differences in input
parameters for physiological model for perchloroethylene." *Environmental Research* 67: 54-67
(1994).

Use of Tissue Disposition Data from Rats and Dogs to Determine Species Differences in Input Parameters for a Physiological Model for Perchloroethylene

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Tissue disposition of perchloroethylene (PCE) was determined experimentally in two mammalian species of markedly different size in order to derive input parameters for the development of a physiologically based pharmacokinetic (PBPK) model, which could forecast the disposition of PCE in each species. Male Sprague-Dawley rats and male beagle dogs received a single bolus of 10 mg PCE/kg body wt in polyethylene glycol 400 by gavage. Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, perirenal fat, and blood were taken for up to 72 hr following PCE administration. Blood and tissue PCE concentrations were analyzed using a gas chromatography headspace technique. Dogs exhibited considerably longer tissue and blood half-lives than did rats. The dogs also exhibited larger area under tissue concentration versus time curves for all tissues except the liver. Whole body clearance of PCE in the rat was greater than that in the dog. Model simulations indicated this could be attributed to more rapid and extensive PCE exhalation and metabolism by the rat. The *in vivo* blood:air partition coefficient determined for rats was similar to an *in vitro* value previously reported. *In vivo* tissue: blood partition coefficients, however, were 1.4 to 2.8 times greater than published *in vitro* values. The PCE *in vivo* blood:air partition coefficient for the dog was twice that of the rat, but tissue:blood partition coefficients were 1.5 to 3.0 times greater in the rat than in the dog. These results demonstrated the existence of significant differences in partition coefficients in two species commonly used in toxicity testing. The PBPK model was shown to have utility in predicting the impact of metabolism and exhalation on pharmacokinetics of PCE in different species of widely differing size. © 1994

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INTRODUCTION

Physiologically based pharmacokinetic (PBPK) models are being increasingly used in health risk assessments of chemicals, particularly in interspecies extrapolations of pharmacokinetic and toxicologic data. By use of physiological and metabolic parameters for a particular species, accurate predictions of chemical concentrations in the blood and tissues of different species are feasible with PBPK models. Similarities in the anatomy and physiology of mammals make scaling from one species to another possible when experimentally derived physiological data are not available for a species of interest (Dedrick, 1973; Boxenbaum, 1984; Travis, 1987).

Tetrachloroethylene, or perchloroethylene (PCE), a commonly used volatile organic compound (VOC), has frequently received the attention of PBPK modelers. Ward *et al.* (1988) developed a PBPK model for PCE with four tissue compartments. This model adequately predicted empirical data other researchers

compiled in studies of mice (Schumann *et al.*, 1980; Buben and O'Flaherty, 1985), rats (Pegg *et al.*, 1979), and humans (Stewart *et al.*, 1961; Ikeda *et al.*, 1972; Fernandez *et al.*, 1976; Monster *et al.*, 1979). The observed data consisted of PCE concentrations measured in exhaled breath over time and various measures of PCE metabolism. A similar approach was used by Travis (1987) for forecasts of PCE kinetics in rats and humans and by Chen and Blancato (1987) for mice, rats, and humans. There have been descriptions of the utility of PBPK models in predicting metabolite formation following PCE exposure of mice, rats, and humans (Bogen and McKone, 1988; Bois *et al.*, 1990; Hattis *et al.*, 1990). The focus of these studies was not to verify the accuracy of model predictions of concentrations of PCE or its metabolites in target tissues, but to evaluate the models' potential to simulate PCE metabolism for use in cancer risk assessments. Tissue concentrations were not employed in the development or validation of any of the aforementioned PBPK models for PCE. There have not been any PBPK models for PCE or other VOCs published in which the dog was a test species.

Tissue:blood partition coefficients are an important input parameter for PBPK models. These parameters describe the transfer of chemical between the blood and each of the tissue compartments included in the model. Ward *et al.* (1988), Bois *et al.* (1990), and Travis (1987) all utilized partition coefficients derived using an *in vitro* vial equilibration technique (Gargas *et al.*, 1989). An *in vivo* approach to derive tissue:blood partition coefficients for PBPK models has been described (Gallo *et al.*, 1987) in which the tissue-concentration time courses of the test chemical are employed. There has been little opportunity for the use of this procedure because of the paucity of detailed tissue concentration versus time data for most VOCs, including PCE.

Therefore, the time course of uptake, deposition, and elimination of PCE in blood and seven tissues was determined in two species, and the data were utilized to derive partition coefficients for a PBPK model for PCE. The rat and the dog were selected in order to utilize dissimilar species commonly employed in toxicological and pharmacological testing. A PBPK model was used to account for differences in the kinetics of PCE in two species by simulating metabolism and exhalation of PCE in each animal.

METHODS

Male beagle dogs (6–15 kg) obtained from Marshall Farms (North Rose, NY), and male Sprague–Dawley rats (325–375 g) obtained from Charles River Laboratories (Raleigh, NC), were employed in these studies. The animals were maintained on a constant light–dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. Rats were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow were provided *ad libitum*. The rats were used after at least a 7-day acclimation period, at which time they were approximately 12 weeks old. Dogs were housed in dog runs, allowed a 14-day acclimation period, and fed Purina Dog Chow. Dogs used were between 6

and 9 months of age and in a weight range of 6–15 kg. Solvent exposures were initiated between 1000 and 1200 hr each day.

PCE (tetrachloroethylene) of 99+ % purity was obtained from Aldrich Chemical Co. (Milwaukee, WI). The purity of the chemical was verified by gas chromatography.

All animals employed in the tissue kinetic studies were unrestrained and unanesthetized. PCE was administered as a single bolus by gavage in a dose of 10 mg/kg using polyethylene glycol 400 (1 ml/kg body wt) as a vehicle. Groups of three dogs and four rats each were serially sacrificed (using pentobarbital and CO₂ to terminate dogs and rats, respectively) at the following times postdosing: 1, 5, 10, 15, 30, and 60 min and 2, 4, 6, 12, 18, 36, 24, 30, 48, and 72 hr for rats, and 1, 4, 12, 24, 48, and 72 hr for dogs. Blood samples were obtained by cardiac puncture. Approximately 1-g samples of liver, kidney, brain, lung, heart, perirenal fat, and skeletal muscle were then quickly removed and placed into previously chilled 20-ml glass vials containing 2 ml of ice-cold saline and 8 ml of isoctane. These samples were analyzed by headspace gas chromatography using the method developed by Chen *et al.* (1993). Each tissue was homogenized for an established time interval with an Ultra-Turrax SDT homogenizer (Tekmar Co., Cincinnati, OH). These times were kept as brief as possible in order to minimize loss of the halocarbon by volatilization. Brain, liver, and fat were the most easily homogenized, requiring only 3 or 4 sec. Kidney, lung, and heart required 5–8 sec. Skeletal muscle was the most difficult to homogenize, as it required 20 sec. The homogenates were then centrifuged at 1800g for 10 min at 4°C in capped vials. An aliquot of the isoctane layer was transferred to 8-ml headspace vials. These sample vials were capped immediately with rubber septa and a spring washer and crimped to ensure an airtight seal.

A Sigma Model 300 gas chromatograph (GC) equipped with a HS-6 headspace sampler (Perkin Elmer Co., Norwalk, CT) was used for the analysis of the halocarbon. The GC was equipped with an electron capture detector. Analyses were carried out on stainless-steel columns (182 cm × 0.317 cm) packed with 10% FFAP (Alltech Associates, Deerfield, IL). The GC operating conditions were: headspace sampler temperature, 90°C; injection port temperature, 200°C; column temperature, 110°C; detector temperature, 400°C; flow rate for argon/methane carrier gas, 60 ml/min. All analyses were conducted using a 20-μl aliquot of isoctane in the 8-ml headspace vials. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 sec with the carrier gas, and injected automatically by a solenoid-controlled mechanism into the GC column. These conditions resulted in vaporization of the halocarbons in the sample vials, since PCE was heated to a temperature slightly below its boiling point. The vial was subsequently pressurized and vented into the GC. The limit of detection for PCE was approximately 1 ng, or 6.7 parts of PCE per billion parts of air in the sample vial.

Areas under concentration-time curves (AUCs) for blood and tissues were determined from the time of administration to infinity. Total body clearance in each species was calculated by dividing the dose of PCE by the blood AUC. The

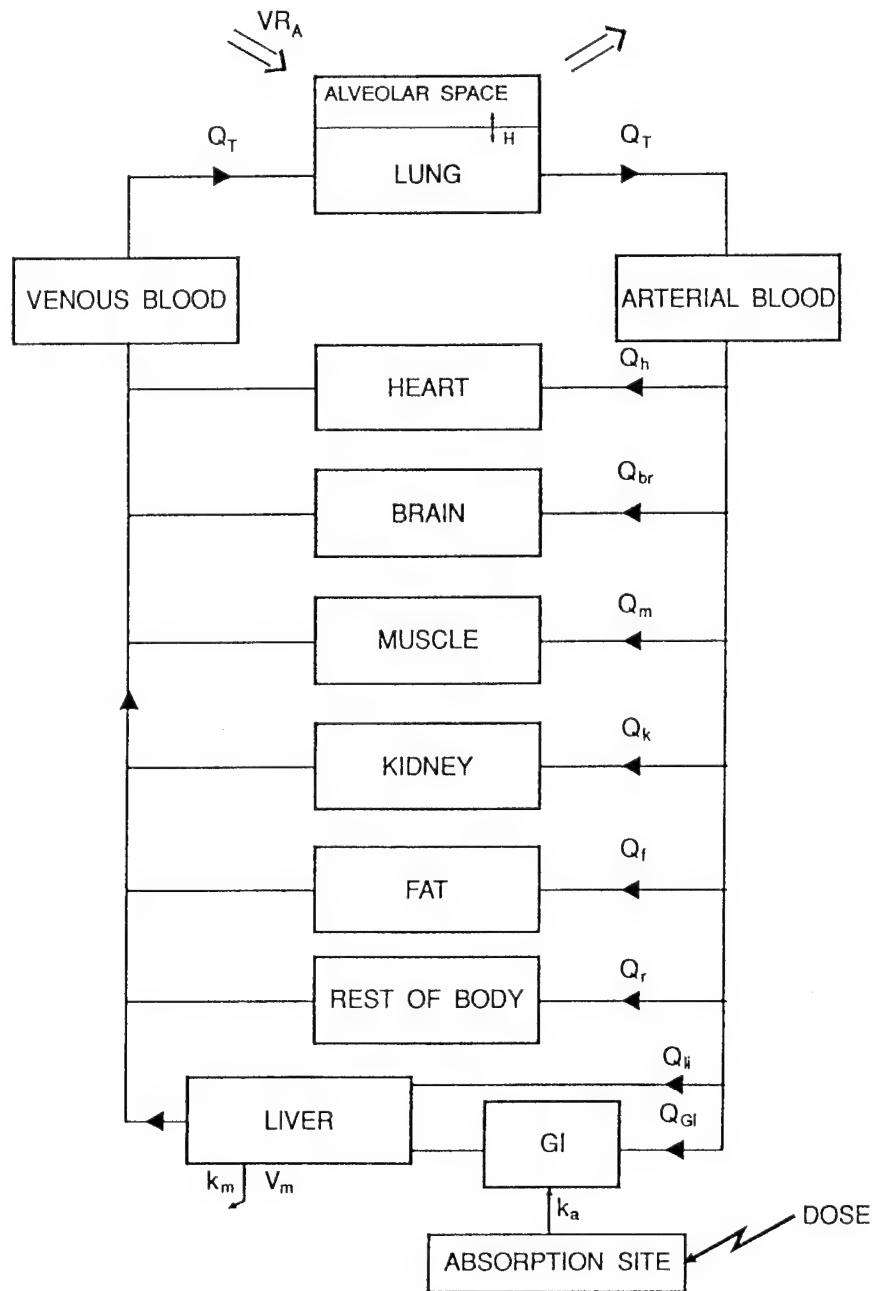


FIG. 1. Diagram of the physiological pharmacokinetic model used to simulate the metabolism and exhalation of PCE following its oral administration to rats and dogs. The parameters used for input into the model are included in Table 1.

maximum PCE concentration reached in blood and tissues (C_{\max}) and the time after dosing that it occurred (T_{\max}) were determined by observation of the available data points. The terminal elimination half-life ($t^{1/2}$) was determined according to the formula: $0.693/\beta$, where β is the terminal elimination rate constant.

Metabolism and exhalation of PCE in the rat and the dog were predicted using a PBPK model for oral administration of PCE (Fig. 1). The model was similar to PBPK models previously developed by Angelo and Pritchard (1984) and Ramsey and Anderson (1984) for other VOCs in that it provided for inhalation and exhalation through a lung compartment, metabolism in a liver compartment, and blood-flow-limited organ representations. The current model differed primarily in that it included a greater number of tissue compartments. Values determined experimentally in this laboratory were employed for tissue volumes (Manning *et al.*, 1991), blood flows (Delp *et al.*, 1991), and alveolar ventilation (Dallas *et al.*, 1991) for the male Sprague-Dawley rat. *In vivo* tissue:blood partition coefficients were calculated from tissue and blood concentration-time data for the rat and the dog by the area method of Gallo *et al.* (1987). For the eliminating organs, non-linear regression analysis was used to determine: (a) the blood:air and lung:blood partition coefficients from the measurements of PCE in the blood and lung; and (b) the liver:blood partition coefficient, K_m , and V_{\max} from the liver and blood PCE data. The absorption rate constant (K_a) was estimated from the data by fitting of the blood and liver concentration versus time data for both species. Tissue volumes and blood flows for the dog were obtained from the literature. Sources of dog tissue volumes were as follows: liver, heart, lung, skeletal muscle, and blood (Andersen, 1970), kidney (Spector, 1956), and fat (Sheng and Huggins, 1971). Sources of blood flows were as follows: liver (Liang *et al.*, 1982), kidney, muscle, and brain (Humphrey and Zins, 1983), cardiac output (Andersen, 1970), fat (Nagasaka *et al.*, 1976), and heart (Liard *et al.*, 1982). Values for alveolar ventilation in the dog were taken from the publication by Andersen (1970).

Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport of PCE in the rat and the dog were numerically integrated with the Advanced Continuous Simulation Language computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted PCE concentrations over time.

RESULTS

Pharmacokinetic parameter estimates for oral administration of PCE to rats are included in Table 2, while blood and tissue PCE concentration versus time profiles are presented in Figs. 2 and 3. After oral dosing, the C_{\max} was reached in 10 to 15 min for each nonlipoidal tissue, other than lung and muscle, which required 60 min. Uptake into the adipose tissue was quite slow (Fig. 3C), in that its T_{\max} was 360 min. As would be anticipated for a chemical as highly lipophilic as PCE, the C_{\max} , AUC, and $t^{1/2}$ values for adipose tissue were substantially greater than those for other tissues. The $t^{1/2}$ for nonfat tissues were relatively consistent, though liver and muscle exhibited somewhat shorter $t^{1/2}$'s.

The rate of blood perfusion and lipid content of individual tissues of rats had a significant influence on PCE deposition in organs. Highly perfused, lipid-rich

TABLE 1

PARAMETERS USED IN THE PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR PCE IN THE RAT AND THE DOG

Parameter	Rat (0.340 kg Body wt)	Dog (10 kg Body wt)
Tissue volumes ^a (ml)		
Liver	3.4	4.2
Kidney	0.8	0.6
Fat	5.0	15.2
Heart	0.3	1.1
Lung	0.4	0.7
Muscle	35.4	46.8
Brain	0.6	0.8
Blood	7.4	8.2
Rest of body	46.8	22.4
Alveolar ventilation ^b	1.54 (ml/min · g) Body wt (g) ^{0.75}	2.58 (ml/min · g) Body wt (g) ^{0.75}
Cardiac output ^c	1.57 (ml/min · g) Body wt (g) ^{0.75}	1.05 (ml/min · g) Body wt (g) ^{0.75}
Blood flows ^c		
Liver	15.7	5.1
Kidney	13.1	10.1
Fat	6.6	5.0
Heart	4.7	3.4
Lung	100	100
Muscle	26.1	40.6
Brain	2.2	3.1
Blood	100% = 1.57 (ml/min · g) Body wt (g) ^{0.75}	100% = 2.05 (ml/min · g) Body wt (g) ^{0.75}
Rest of body	31.5	32.7
Partition coefficients		
Blood:air	19.6	40.5
Fat:blood	150.5	71.4
Lung:blood	1.9	1.3
Liver:blood	5.0	2.4
Muscle:blood	2.4	2.4
Brain:blood	4.1	4.1
Heart:blood	2.4	2.4
Kidney:blood	3.2	2.1
Rest of body:blood	3.0	1.9
Metabolism constants		
V_{max} (μg/min)	0.15	0.85
K_m (μM/ml)	0.019	0.023
Absorption constant		
K_a (min ⁻¹)	0.025	0.34

^a Tissue volumes for rats were determined by Manning *et al.* (1991). Tissue volumes for dogs were obtained from Andersen (1970) for liver, heart, lung, skeletal muscle, and blood; from Spector (1956) for kidney; and from Sheng and Huggins (1971) for fat.

^b Alveolar ventilation values for rats and dogs were obtained from Dallas *et al.* (1991) and from Andersen (1970), respectively.

^c Tissue blood flows for rats were obtained from Delp *et al.*, 1991. Blood flows for dogs were obtained from Liang *et al.* (1982) for liver; from Humphrey and Zins (1983) for kidney, muscle, and brain; from Andersen (1970) for cardiac output; from Nagasaka *et al.* (1976) for fat; and from Laird *et al.* (1982) for the heart.

TABLE 2
PHARMACOKINETIC PARAMETERS IN THE RAT FOLLOWING ORAL ADMINISTRATION OF 10 mg
PCE/kg BODY WT^a

Tissue	Area under curve ($\mu\text{g} \cdot \text{min}/\text{ml}$)	Half-life (min)	C_{\max} ($\mu\text{g/g}$)	T_{\max} (min)
Liver	1,673 \pm 345	331 \pm 2	12.3 \pm 7.6	10
Kidney	1,057 \pm 139	395 \pm 60	5.5 \pm 3.1	10
Fat	49,964 \pm 9,745	695 \pm 154	36.0 \pm 17.4	360
Heart	806 \pm 145	396 \pm 73	2.9 \pm 0.4	15
Lung	627 \pm 167	342 \pm 69	1.6 \pm 0.8	60
Muscle	798 \pm 428	310 \pm 37	2.1 \pm 0.8	60
Brain	1,377 \pm 230	327 \pm 35	5.1 \pm 0.8	15
Blood	332 \pm 145	384 \pm 145	1.0 \pm 0.2	15

^a Each value represents the mean \pm SD for four rats at 14 time points ranging from 1 min to 72 hr.

organs, such as the liver and brain, had quite high C_{\max} and AUC values. Although not lipid-rich, the highly perfused kidneys also exhibited relatively high C_{\max} and AUC values. Poorly perfused, nonlipoidal tissues, such as skeletal muscle (Fig. 3B), had relatively low C_{\max} and AUC values.

Pharmacokinetic parameter estimates for dogs following oral administration of PCE are presented in Table 3, while blood and tissue level time courses are shown

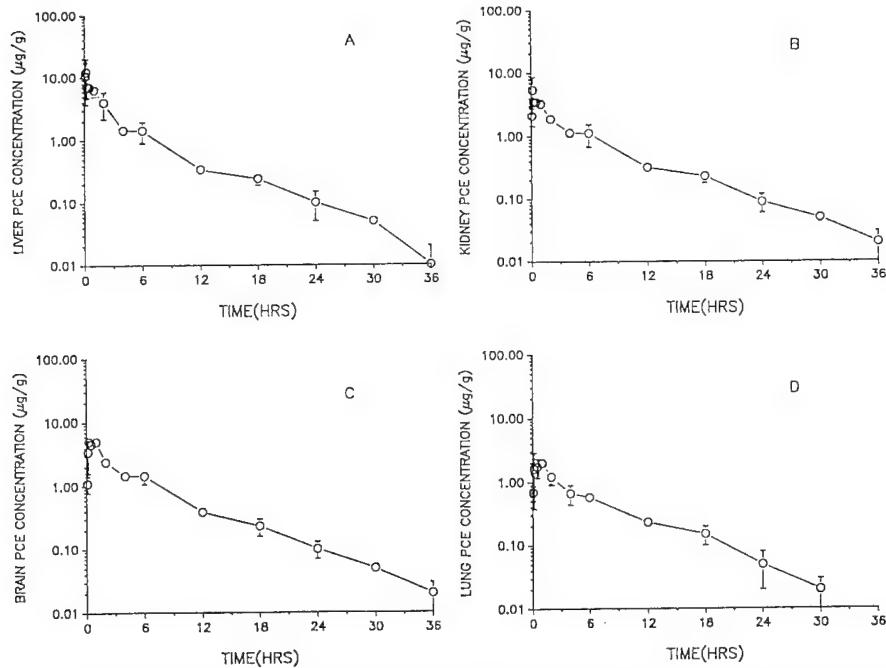


FIG. 2. PCE concentrations measured in the liver (A), kidney (B), brain (C), and lung (D) of rats following administration of a single bolus of 10 mg/kg of PCE by gavage. Each point represents the mean \pm SD for four rats.

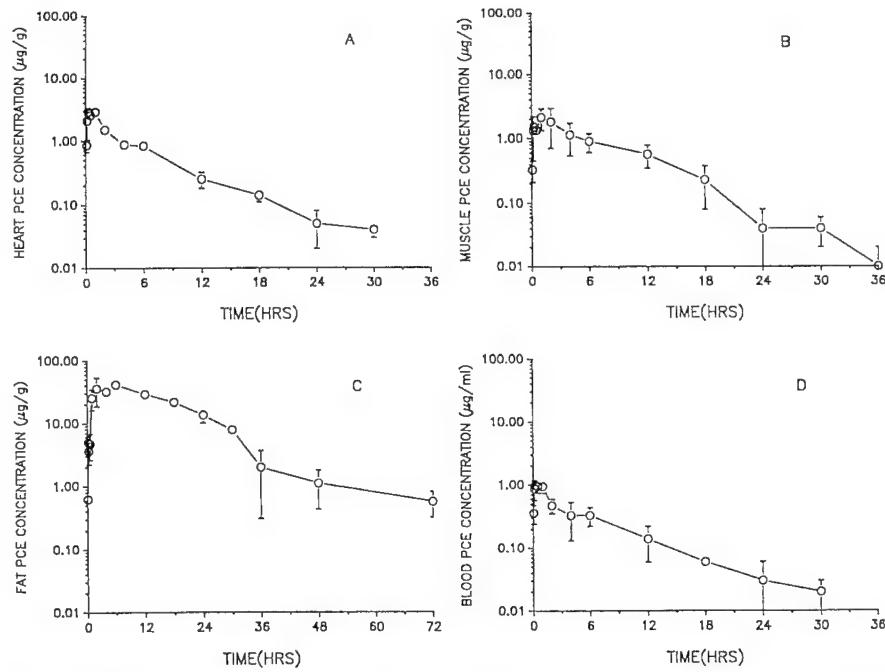


FIG. 3. PCE concentrations measured in the heart (A), muscle (B), fat (C), and blood (D) of rats following administration of a single bolus of 10 mg/kg of PCE by gavage. Each point represents the mean \pm SD for four rats.

in Figs. 4 and 5. The observed T_{\max} for all nonlipoidal tissues was at the first sampling point (i.e., 60 min). The actual T_{\max} 's for these tissues were probably shorter, though this cannot be ascertained from the present study, since no earlier sampling times were employed. The T_{\max} for fat in the dog was also twice as long as that in the rat, though the C_{\max} 's in the two species were comparable. As in the rat, much greater deposition of PCE was observed in the fat of the dog than in the

TABLE 3
PHARMACOKINETIC PARAMETERS IN THE DOG FOLLOWING ORAL ADMINISTRATION OF 10 mg
PCE/kg BODY WT^a

Tissue	Area under curve ($\mu\text{g} \cdot \text{min}/\text{ml}$)	Half-life (min)	C_{\max} ($\mu\text{g}/\text{g}$)	T_{\max} (min)
Liver	1,851 \pm 757	2448 \pm 922	6.3 \pm 0.6	60
Kidney	1,606 \pm 621	1572 \pm 262	4.9 \pm 0.4	60
Fat	55,838 \pm 9,640	494 \pm 77	42.8 \pm 3.5	720
Heart	1,849 \pm 620	1775 \pm 464	5.7 \pm 1.6	60
Lung	1,001 \pm 681	2289 \pm 863	2.4 \pm 0.5	60
Muscle	1,907 \pm 1,564	1625 \pm 886	3.1 \pm 0.1	60
Brain	3,238 \pm 1,153	4641 \pm 1547	11.4 \pm 8.2	60
Blood	782 \pm 146	865 \pm 385	1.5 \pm 0.3	60

^a Each value represents the mean \pm SD for three dogs at six time points ranging from 1 to 72 hr.

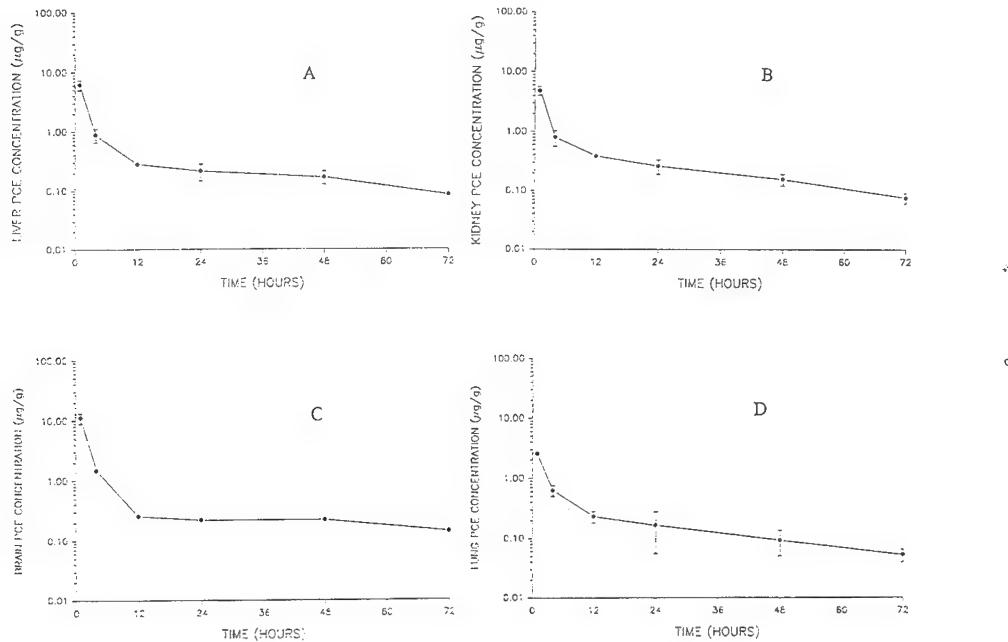


FIG. 4. PCE concentrations measured in the liver (A), kidney (B), brain (C), and lung (D) of dogs following administration of a single bolus of 10 mg/kg of PCE by gavage. Each point represents the mean \pm SD for three dogs.

other tissues (Fig. 5C). Except for the brain, C_{\max} and AUC values for the fat were at least 8 and 33 times higher, respectively, than in nonfat tissues. PCE accumulation in the dog brain was considerably higher than that in the other nonfat tissues (Table 3). Unlike the rat, the other highly perfused tissues (i.e., kidney and liver) of the dog did not show C_{\max} and AUC values that were different from less well-perfused, lean tissues. For the C_{\max} values, this is likely due to the lack of available time points during the first hour. The $t_{1/2}$ for the dog brain was significantly longer than that for other tissues, whereas the $t_{1/2}$ for the rat brain was similar to that for most other tissues. The liver $t_{1/2}$, which was relatively short in rats, was comparable to a number of other tissues in the dog.

PCE tissue dosimetry varied substantially in the dog and the rat. AUCs were larger for dog tissues than for corresponding rat tissues, except for liver. The larger AUCs in dog tissues can be attributed primarily to prolonged elimination, since C_{\max} values were generally comparable in each species for most tissues. The $t_{1/2}$ in the nonfat dog tissues were 4–12 times longer than those in corresponding rat tissues. The apparently short $t_{1/2}$ value for dog fat was due to a lack of time points after 72 hr, such that an accurate $t_{1/2}$ could not be determined. Following oral administration, whole body clearance of PCE was 30.1 and 12.8 ml min/kg for rats and dogs, respectively.

The time courses of exhalation and metabolism of PCE were predicted by the PBPK model for the rat (Fig. 6A) and dog (Fig. 6B) following oral administration of the chemical. For each species, the fraction of PCE exhaled was substantially

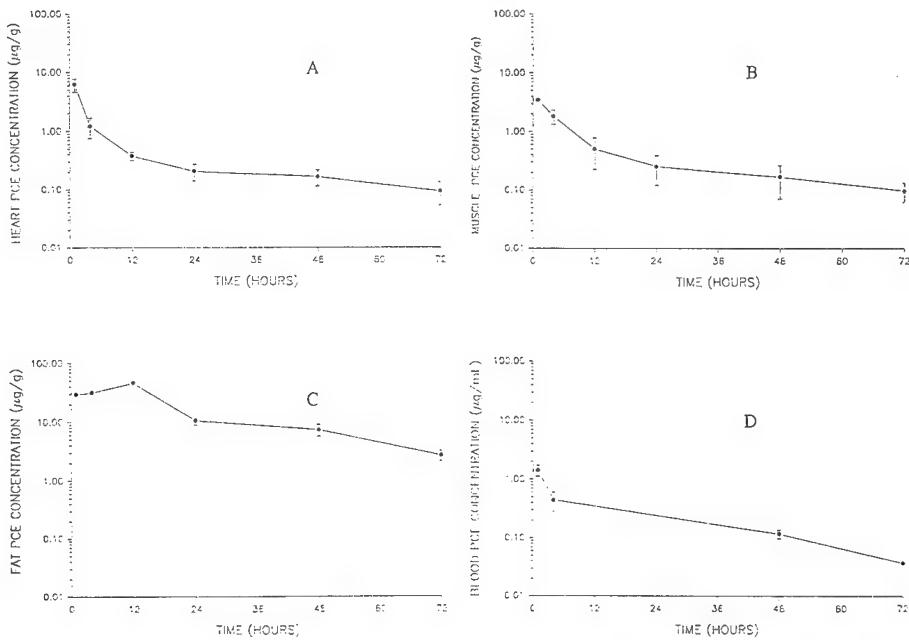


FIG. 5. PCE concentrations measured in the heart (A), muscle (B), fat (C), and blood (D) of dogs following administration of a single bolus of 10 mg/kg of PCE by gavage. Each point represents the mean \pm SD for three dogs.

greater than the fraction metabolized, with the difference steadily increasing during the period following oral dosing. The rate and magnitude of exhalation and metabolism were markedly higher in the rat than in the dog.

DISCUSSION

Tissue concentration-time data were obtained in two species in this study in order to derive *in vivo* partition coefficients to utilize in development of a PBPK model for PCE. Previously, such detailed tissue concentration-time data sets have

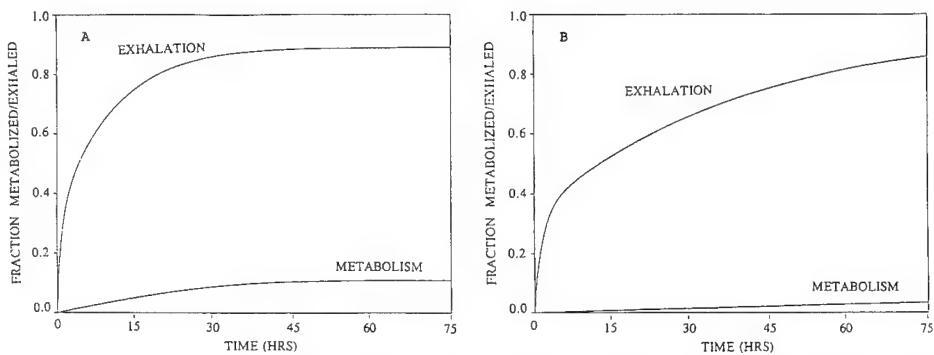


FIG. 6. Model predictions of the exhalation and metabolism of PCE over time, following po dosing of (A) rats and (B) dogs, with a single 10 mg/kg bolus dose of PCE.

not been available for VOCs, including PCE. The most logical measure of target organ exposure to toxicants is the AUC versus time curve of the biologically active form of a chemical (Andersen, 1987). The AUCs measured in the present study, following oral administration of PCE to rats and dogs, have proven useful in verifying assumptions concerning the interspecies scaling of tissue deposition. Interspecies scaling has been described as the determination of how target tissue exposure is affected by the size of a species for a particular administered dose (Andersen, 1987). By scaling metabolic and physiological clearance as a power of body weight and tissue volumes in proportion to body weight, the NRC (1986) predicted that greater AUCs for parent compounds would be manifest in larger animals. Findings in the current study support this concept. AUCs were greater in dogs than in rats in six of seven tissues following ingestion of an equal oral dose of PCE. Andersen (1987) concluded that since clearance increases as a fractional power of body weight, it will be greater in smaller species. Indeed, clearance of PCE is much greater in rats than in dogs in the present investigation. Blood and tissue $t_{1/2}$ values were much shorter in rats than in dogs (Tables 2 and 3). The major route of PCE elimination is exhalation (Pegg *et al.*, 1979; Schumann *et al.*, 1980). The blood:air partition coefficient is smaller in rats (19.8) than in dogs (40.5), so PCE will more readily diffuse from the pulmonary blood into the alveolar air of the rat. The rate of PCE exhalation is also dependent upon the rates of pulmonary (blood) perfusion and alveolar ventilation, both of which are significantly higher in the rat. Although metabolism plays a limited role in systemic clearance of PCE, metabolism would be expected to be greater in rats. Both exhalation and metabolism of PCE were predicted to be higher in the rat than in the dog by the PBPK model employed in the current investigation.

The primary method that has been employed to date to derive partition coefficients for VOCs has been the vial equilibration technique (Gargas *et al.*, 1989). This *in vitro* technique is based on a similar method previously used to estimate partition coefficients for blood, oil, and water (Sato and Nakajima, 1979) and tissue homogenates (Fiserova-Bergerova *et al.*, 1984). The blood:air partition coefficient for PCE reported by Gargas *et al.* (1989) (18.9) was quite similar to that calculated from *in vivo* data for rats in the current study (19.8). Our *in vivo* tissue:blood partition coefficients, however, were consistently higher than the limited number of *in vitro* values published by Gargas *et al.* (1989) for rats. Corresponding *in vivo* and *in vitro* tissue:blood partition coefficients were as follows: liver:blood, 5.0 and 3.71; muscle:blood, 2.4 and 1.06; and fat:blood, 150.5 and 86.7. In contrast, the tissue:blood partition coefficients were 1.5 to 3.0 times higher in the rat than in the dog. As the *in vivo* partition coefficients are derived from the tissue AUCs, it should be recognized that errors in determination of these values will be reflected in the accuracy of the coefficients. For example, since PCE levels were relatively high at the last sampling time (i.e., 72 hr), an inaccurate estimation of the elimination rate constant could compromise the validity of the fat:blood partition coefficient. Although Gargas *et al.* (1989) reported similar PCE blood:air partition coefficients for mice and rats, the values were more than 75% higher than those for humans. In the current study, the blood:air coefficient for the dog was more than twice that of the rat. These results demon-

strate the importance of accurately determining and recognizing species differences in partition coefficients.

In an evaluation of the uncertainties involved in PBPK models for PCE, Hattis *et al.* (1990) found that there was appreciable variance in predictions of models of different researchers. Of the many potential sources of uncertainty, it was concluded that the primary cause of differences in model predictions of risk were the approaches employed in estimating the metabolic parameters. There were 20- and 60-fold differences in estimates of K_m for mouse and rat models, respectively. V_{max} estimates differed by 8- and 15-fold for the mouse and rat models, respectively. The values for K_m and V_{max} in the present study were estimated by non-linear regression, fitting the model predictions to the observed blood and liver concentration versus time data. Our V_{max} value (2.9 nM/ml kg) for rats, when expressed in the same units, is 10 times lower than the range of values evaluated by Hattis *et al.* (1990). The range of estimates of V_{max} reported by Hattis *et al.* (1990) was not based on liver concentration-time data. Due to the dependence of PBPK models on a number of variables, there are other potential sources of error including model structure and other input parameters.

It has frequently been necessary during the development of the PBPK models for VOCs to manipulate anatomical, physiological, and physicochemical input parameters in order to obtain adequate simulations of observed/experimental data. In an analysis of the precision of PBPK models for PCE in risk assessment, Bois *et al.* (1990) quantified the uncertainties associated with parameter variability. The kinetic parameter defining metabolic rate was seen as the most important variable in assessing cancer risk. Changes in key input parameters have often had to be made arbitrarily because of incorrect or inadequate information. Ramsey and Andersen (1984), for example, had to make the following changes in their PBPK model for styrene to obtain good agreement between observed and predicted values: increase in the fat tissue volume and blood perfusion rate; decrease in the fat:blood partition coefficient; increase of the blood flow rate to the metabolizing tissue group; and offsetting alterations in the muscle and richly perfused tissue groups. Reitz *et al.* (1988) found that the pharmacokinetics of trichloroethane in older rats could be simulated by increasing the size of the fat compartment in their model. It has frequently been necessary to make such assumptions and utilize unverified values for input parameters in the absence of data. Determination and utilization of accurate parameters should significantly improve the accuracy of PBPK model simulations. Therefore, *in vivo* studies that provide species-specific physiological and physicochemical parameters, as was done for dogs and rats in the present investigation, can significantly reduce uncertainty of PBPK model predictions of the systemic disposition of VOCs.

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APPENDIX F

Dallas, C.E., Chen, X.M., Muralidhara, S., Varkonyi, P., Tackett, R.L., and Bruckner, J.V. "Physiologically based pharmacokinetic model useful in predictions of the influence of species, dose, and exposure route on perchloroethylene pharmacokinetics." *Journal of Toxicology and Environmental Health* **44**: 301-317 (1995).

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL USEFUL IN PREDICTION OF THE INFLUENCE OF SPECIES, DOSE, AND EXPOSURE ROUTE ON PERCHLOROETHYLENE PHARMACOKINETICS

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The ability of a physiologically based pharmacokinetic (PBPK) model to predict the uptake and elimination of perchloroethylene (PCE) in venous blood was evaluated by comparison of model simulations with experimental data for two species, two routes of exposure, and three dosage levels. Unanesthetized male Sprague-Dawley rats and beagle dogs were administered 1, 3, or 10 mg PCE/kg body weight in polyethylene glycol 400 as a single bolus, either by gavage or by intraarterial (ia) injection. Serial blood samples were obtained from a jugular vein cannula for up to 96 h following dosing. The PCE concentrations were analyzed by headspace gas chromatography. For each dose and route of administration, terminal elimination half-lives in rats were shorter than in dogs, and areas under the blood concentration-time curve were smaller in rats than in dogs. Over a 10-fold range of doses, PCE blood levels in the rat were well predicted by the PBPK model following ia administration, and slightly underpredicted following oral administration. The PCE concentrations in dog blood were generally overpredicted, except for fairly precise predictions for the 3 mg/kg oral dose. These studies provide experimental evidence of the utility of the PBPK model for PCE in interspecies, route-to-route, and dose extrapolations.

Knowledge of the influence of different species, dose levels, and routes of administration on the kinetics of environmental chemicals has been considerably enhanced by the use of physiologically based pharmacokinetic (PBPK) models (Clewell & Andersen, 1985; Reitz et al., 1988). Similarities in the anatomy and physiology of mammalian species make interspecies extrapolations of pharmacokinetic data (i.e., animal scale-up or -down) possible (Dedrick, 1973; Boxenbaum, 1984). Pharmacokinetic models developed in one species may be scaled, on the basis of allometric relationships, to allow prediction of chemical concentrations in other species. Model input

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parameters such as alveolar ventilation, tissue volumes and blood flows, and metabolism can be scaled from one species to another. Changing the amount of chemical entering the animal allows for extrapolations between doses. Altering the point of entry of the chemical into the model enables pharmacokinetic comparisons between routes of administration.

The PBPK models have been used to assess the effect of dose of PCE on the rate of urinary metabolite formation in mice and humans (Ward et al., 1988; Bois et al., 1990). The influence of different routes of exposure on PCE pharmacokinetics has also been evaluated using a PBPK model (Travis, 1987). Thus far, mice, rats, and humans have been the species employed in most interspecies comparisons and extrapolations employing PBPK models for PCE (Ward et al., 1988; Bois et al., 1990; Chen & Blancato, 1987). In most instances, model simulations of PCE exhalation and metabolism were compared to published data of other investigators. At this time, there have not been adequate data available on the time course of blood PCE levels in rats to allow one to evaluate the accuracy of PBPK model predictions. No one has published and validated a PBPK model for volatile organic chemicals (VOCs), including PCE, in the dog, despite the widespread use of this animal in pharmacokinetic and toxicology studies.

Another primary need in the development of PBPK models has been the accurate derivation of the tissue-blood partition coefficients, which delineate the rate and magnitude of the movement of the chemical between the blood and the model's tissue compartments. Previously published model predictions for PCE (Travis et al., 1987; Ward et al., 1988; Bois et al., 1990; Byczkowski et al., 1994) and other VOCs have employed partition coefficients that were derived using an in vitro vial equilibration technique (Gargas et al., 1989). Recently, tissue-blood partition coefficients for a PBPK model for PCE were derived from in vivo data (Dallas et al., 1994), using a method that employs area under the tissue concentration (AUTC) time-course data (Gallo et al., 1987). It is feasible that the use of these in vivo-derived partition coefficients may provide an increased level of certainty in the utility of the models in making accurate predictions across species, doses, and routes of administration.

The objective of the current study, therefore, was to evaluate the efficacy of this new PBPK model with in vivo-derived partition coefficients to forecast the kinetics of PCE in the rat and dog. The ability of the model to predict blood PCE levels in animals given different doses by different routes of exposure was also evaluated, by comparison of experimental blood concentration time courses with model simulations.

METHOD

Male beagle dogs (5–10 kg), obtained from Marshall Farms (North Rose, N.Y.), and male Sprague-Dawley rats (325–375 g), obtained from Charles River Laboratories (Raleigh, N.C.), were employed in these studies. The ani-

mals were maintained on a constant light-dark cycle, with light from 0700 to 1900 h and darkness from 1900 to 0700 h. Rats were housed in polypropylene cages in an animal rack. Tap water and Ralston Purina Formulab Chow were provided ad libitum. The rats were used after at least a 7-d acclimation period, at which time they were approximately 12 wk old. Dogs were housed in runs and were fed Purina Dog Chow during a 14-d acclimation period. Dogs were used when they were between 6 and 9 mo of age and in a weight range of 6–15 kg. Solvent exposures were initiated between 1000 and 1200 h each day.

1,1,2,2-Perchloroethylene (PCE; tetrachloroethylene) of 99%+ purity was obtained from Aldrich Chemical Co. (Milwaukee, Wis.). The purity of the chemical was verified by gas chromatography.

An indwelling carotid arterial cannula was surgically implanted into rats and dogs the day before intraarterial (ia) administration of PCE. An indwelling jugular vein cannula was also implanted into all the test animals for serial blood sampling. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml), acepromazine maleate (10 mg/ml), and xylazine HCl (20 mg/ml) in a proportion of 3 : 2 : 1 (v/v/v). Dogs were anesthetized with 50 mg/ml Nembutal. The cannulated animals were allowed freedom of movement during the 24-h recovery period, with the cannulae protected from manipulation by exteriorization and taping at the back of the head. All animals employed in the kinetic studies were unanesthetized. The PCE, in doses of 1, 3, or 10 mg/kg, was administered as a single bolus orally or ia using polyethylene glycol 400 (1 ml/kg body weight) as a vehicle. The ia injections were conducted through the carotid arterial cannula. Oral doses were administered by use of a ball-tipped gavage needle for rats and a Teflon tube for dogs. Food was withheld during the 18-h recovery period before dosing.

Serial 20- μ l blood samples were collected from the indwelling jugular vein cannula at intervals up to 96 h following dosing. While rats exhibited only slight neurobehavioral effects following ia administration of PCE, dogs receiving the 10 mg/kg ia dose demonstrated pronounced central nervous system (CNS) depression. Dog ia data are therefore presented only for the 1 and 3 mg/kg doses. The PCE concentrations in the blood of dogs given 1 mg/kg orally declined rapidly below the limit of detection. Therefore, the data for this group were not plotted nor used to calculate pharmacokinetic parameters.

The PCE concentrations in the blood samples were analyzed by headspace gas chromatography (GC), according to the method developed by this laboratory (Chen et al., 1993). The blood samples were transferred to 8-ml headspace vials. These vials were capped immediately with rubber septa and spring washers and crimped to ensure an airtight seal. Each sample vial was then placed into the autosampler unit of a GC. The limit of detection for PCE was approximately 1 ng, or 6.7 parts of PCE per billion parts of air in the sample vial.

A Sigma model 300 GC equipped with an electron capture detector and an HS-6 headspace sampler (Perkin Elmer Co., Norwalk, Conn.) was used for the analysis of the halocarbon. Analyses were carried out on stainless-steel columns (182×0.317 cm) packed with 10% FFAP (Alltech Associates, Deerfield, Ill.). The GC operating conditions were: headspace sampler temperature, 90°C ; injection port temperature, 200°C ; column temperature, 110°C ; detector temperature, 400°C ; flow rate for argon/methane carrier gas, 60 ml/min. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 s with the carrier gas, and injected automatically by a solenoid-controlled mechanism into the GC column. The aforementioned conditions resulted in vaporization of the halocarbon in the sample vial, since PCE was heated to a temperature slightly below its boiling point.

The disposition of PCE in the rat and the dog was predicted using a PBPK model for po and ia administration (Figure 1). It was similar to PBPK models previously developed (Angelo & Pritchard, 1984; Ramsey & Andersen, 1984) for other VOCs, in that it provided for inhalation and exhalation through a lung compartment, metabolism in a liver compartment, and blood-flow-limited organ representations. The current model differed primarily in that it included a greater number of tissue compartments. For oral administration, the model had a gastrointestinal (GI) compartment, with its rate of blood flow and oral absorption rate (K_a) dictating uptake of the PCE. Values in our laboratory in the male Sprague-Dawley rat were employed for tissue volumes (Manning et al., 1991), blood flows (Delp et al., 1991), and alveolar ventilation (Dallas et al., 1991). In vivo tissue-blood partition coefficients were calculated from tissue concentration-time-course data following po administration to rats and dogs (Dallas et al., 1994), using the area method (Gallo et al., 1987). For the eliminating organs, nonlinear regression analysis was used to determine (a) the blood-air and lung-blood coefficients from the measurement of PCE in the blood and lungs, and (b) the liver-blood partition coefficient, K_m and V_{max} from the liver and blood PCE data. The value for K_a was estimated from oral data by fitting of the blood and liver concentration versus time data for both species. Tissue volumes and blood flows for the dog were obtained from the literature. Sources of dog tissue volumes were as follows: liver, heart, lung, skeletal muscle, and blood (Andersen, 1970), kidney (Spector, 1956), and fat (Sheng & Huggins, 1971). Sources of blood flows in the dog were as follows: liver (Liang et al., 1982), kidney, muscle, and brain (Humphrey & Zins, 1983), cardiac output (Andersen, 1970), fat (Nagasaka et al., 1976), and heart (Liard et al., 1982). Values for alveolar ventilation in the dog were taken from the publication by Andersen (1970).

The blood concentration versus time data were evaluated by the Lagran computer program (Rocci & Jusko, 1983) for the assessment of the appropriate pharmacokinetic model and calculation of pharmacokinetic parameters. The area under the blood concentration-time curve (AUC) was determined

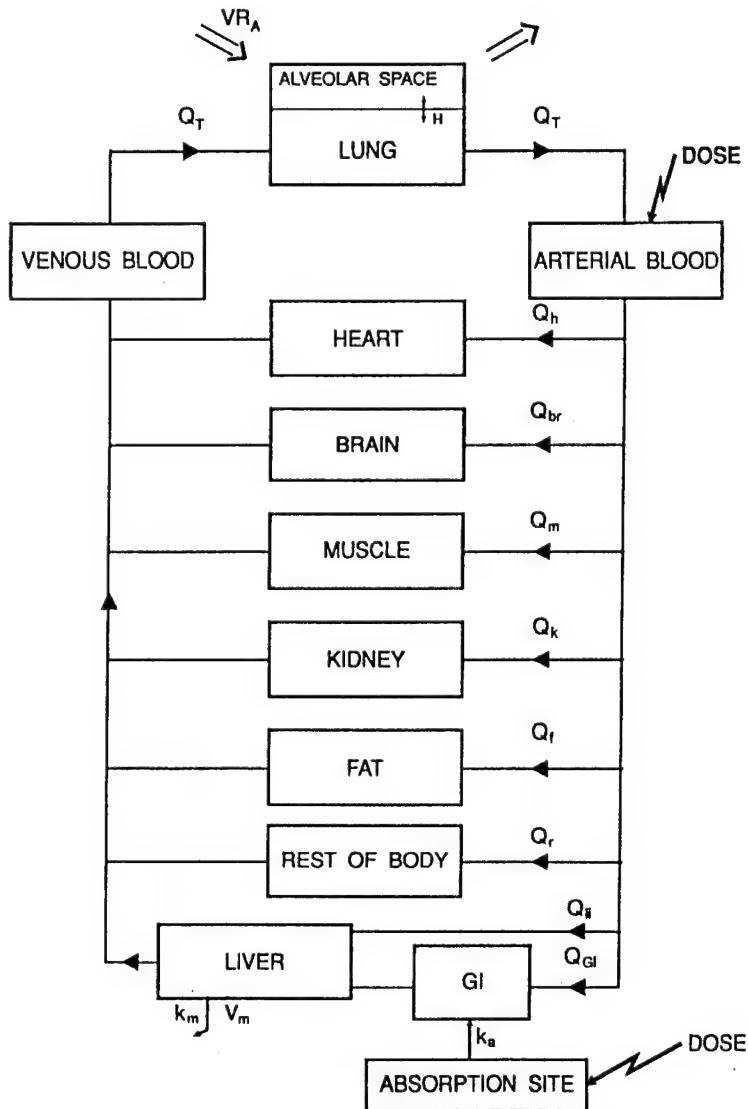


FIGURE 1. Diagram of the physiological pharmacokinetic model used to predict the uptake and elimination of PCE in venous blood following ia or oral administration to rats and dogs. The parameters used for input into the model are included in Table 1.

from the time of administration to infinity. Bioavailability was calculated by AUC_{po}/AUC_{ia} . Total body clearance was determined as the dose divided by the blood AUC in each species. The maximum concentration of PCE reached in blood and tissues (C_{max}) and the time after dosing that it occurred (T_{max}) were determined by observation of the available data points. The terminal elimination half-life ($t_{1/2}$) was determined according to the formula $0.693/\beta$,

where β is the terminal elimination rate constant determined with the computer program. The statistical significance of differences in individual animal pharmacokinetic parameters was determined by Student's *t*-test between the different species, doses, and routes of administration.

Differential mass balance equations, incorporating the parameters listed in Table 1, that describe the transport of PCE in the rat and dog were numer-

TABLE 1. Parameters Used in the Physiologically Based Pharmacokinetic Model for PCE in the Rat and the Dog

Parameter	Rat, 0.340 kg body weight	Dog, 10 kg body weight
Tissue volumes (ml)	Percentage of body weight	
Liver	3.4	4.2
Kidney	0.8	0.6
Fat	5.0	15.2
Heart	0.3	1.1
Lung	0.4	0.7
Muscle	35.4	46.8
Brain	0.6	0.8
Blood	7.4	8.2
Rest of body	46.8	22.4
Alveolar ventilation	$1.54 \text{ (ml/min} \cdot \text{g}) \times \text{BW(g)}^{0.75}$	$2.58 \text{ (ml/min} \cdot \text{g}) \times \text{BW(g)}^{0.75}$
Cardiac output	$1.57 \text{ (ml/min} \cdot \text{g}) \times \text{BW(g)}^{0.75}$	$1.05 \text{ (ml/min} \cdot \text{g}) \times \text{BW(g)}^{0.75}$
Blood flows	Percentage of cardiac output	
Liver	15.7	5.1
Kidney	13.1	10.1
Fat	6.6	5.0
Heart	4.7	3.4
Lung	100	100
Muscle	26.1	40.6
Brain	2.2	3.1
Blood	$100\% = 1.57 \text{ (ml/min} \cdot \text{g}) \times \text{BW(g)}^{0.75}$	$100\% = 2.05 \text{ (ml/min} \cdot \text{g}) \times \text{BW(g)}^{0.75}$
Rest of body	31.5	32.7
Partition coefficients		
Blood-air	19.6	40.5
Fat-blood	152.5	63.2
Lung-blood	2.5	1.0
Liver-blood	5.2	1.6
Muscle-blood	3.0	1.9
Brain-blood	4.4	2.7
Heart-blood	2.7	1.7
Kidney-blood	4.4	1.5
Rest of body-blood	3.0	1.9
Metabolism constants		
V_{max} ($\mu\text{g}/\text{min}$)	0.15	0.85
K_m ($\mu\text{m}/\text{ml}$)	0.019	0.023
Absorption constant K_a (min^{-1})	0.025	0.34

ically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, Mass.). The solution to the equations provided predicted PCE concentrations over time.

RESULTS

Pharmacokinetic parameter estimates for the different doses and routes of administration are shown in Table 2 for rats and Table 3 for dogs. Following oral administration of PCE to rats, maximum blood concentrations (C_{\max}) were achieved in approximately 20–40 min for all three doses. Increases in AUCs were slightly more than proportional to increases in dose for both the ia and po groups, although AUC did not vary as a function of route of administration. Clearance diminished with an increase in dose in the ia-dosed animals, and both the groups receiving the 3 and 10 mg/kg po doses had lower clearance values than the 1 mg/kg group. The $t_{1/2}$ was significantly longer in the po-dosed rats than in those receiving PCE by ia injection at the 3 ($p < .05$) and 10 mg/kg ($p < .01$) dosage-levels. Half-life increased significantly with increase in dose in both the ia and po groups. The PCE was well absorbed from the GI tract, as manifest by high bioavailability values.

Unfortunately, it was difficult to evaluate the dose dependency of PCE kinetics in dogs, due to the inability to obtain complete data sets for each exposure route over the range of doses given. The $t_{1/2}$ values at the one dose (3 mg/kg) for which ia and po data were available were not significantly different. As was seen in rats, clearance decreased as the increase in the ia dose given to dogs. Conversely, clearance increased as oral dose increased from 3 to 10 mg/kg, as the AUC did not increase in proportion to dose in these two groups (Table 3). Bioavailability of PCE was essentially 100% in dogs receiving 3 mg/kg orally.

Marked species differences in PCE pharmacokinetics were apparent upon comparison of kinetic parameters in Tables 2 and 3. For corresponding doses and routes of administration, the AUC in dogs was approximately twice that observed in rats at 1 and 3 mg/kg. Half-life values were significantly longer ($p < .001$) in dogs, except at the highest (10 mg/kg) po dose. The C_{\max} achieved in dogs was approximately 3 times that in rats for equivalent ia and po doses, with the exception of the 10 mg/kg po dose.

Predictions of PCE blood concentrations over the time following ia injection are compared to experimental (i.e., observed) values for the three doses of PCE in the rat in Figure 2. Over the 10-fold range of doses employed, concentrations of PCE in the venous blood were well predicted by the model. Blood PCE concentrations during the initial phase of rapid decline in the blood were slightly overpredicted. Model-generated concentrations were in close agreement, however, with the observed blood levels during the terminal elimination phase for all three doses.

Observed and predicted concentrations of PCE in the venous blood of dogs are shown after ia administration of PCE in Figure 3. Blood PCE con-

TABLE 2. Pharmacokinetic Parameters Estimates for the Rat

Dose (mg/kg)	Route	n	Area under curve ($\mu\text{g} \cdot \text{min}/\text{ml}$)	Biological half-life (h)	C_{\max} ($\mu\text{g}/\text{ml}$)	T_{\max} (min)	Bioavailability (F)	Clearance ($\text{ml}/\text{min}/\text{kg}$)
1	PO	6	27.9 ± 1.7	—	0.2 ± 0.0	20.8 ± 2.0	0.96	36.7 ± 2.8
	IA	6	29.2 ± 2.3	—	0.6 ± 0.2	1.2 ± 0.4		34.8 ± 2.4
	PO	5	100.5 ± 10.8	7.8 ± 1.0	0.4 ± 0.1	37.0 ± 23.9	0.94	30.1 ± 2.9
3	IA	5	107.3 ± 16.8	6.0 ± 1.2	1.3 ± 0.2	2.8 ± 1.6		28.5 ± 4.3
	PO	6	321.4 ± 27.6	15.5 ± 1.5	1.6 ± 0.2	22.5 ± 4.8	0.82	32.5 ± 3.3
10	IA	4	391.6 ± 36.4	7.5 ± 1.7	4.1 ± 0.4	6.0 ± 0.8		26.2 ± 2.5

Note. Each value represents the mean ± SE. For route, PO represents oral administration and IA intraarterial administration.

TABLE 3. Pharmacokinetic Parameters Estimates for the Dog

Dose (mg/kg)	Route	n	Area under curve ($\mu\text{g} \cdot \text{min}/\text{ml}$)	Biological half-life (h)	C_{\max} ($\mu\text{g}/\text{ml}$)	T_{\max} (min)	Clearance ($\text{ml}/\text{min}/\text{kg}$)
1	IA	4	50.3 ± 11.3	—	0.6 ± 0.2	5.5 ± 1.7	23.0 ± 4.7
3	PO	4	214.2 ± 25.4	20.8 ± 2.2	1.2 ± 0.2	15.0 ± 0.0	14.6 ± 1.8
3	IA	3	214.0 ± 17.4	21.6 ± 1.6	4.5 ± 1.0	2.3 ± 0.7	14.3 ± 1.1
10	PO	8	451.1 ± 51.2	16.6 ± 1.4	1.9 ± 0.1	31.0 ± 9.9	25.0 ± 3.9

Note. Each value represents the mean ± SE. For route, PO represents oral administration and IA intraarterial administration.

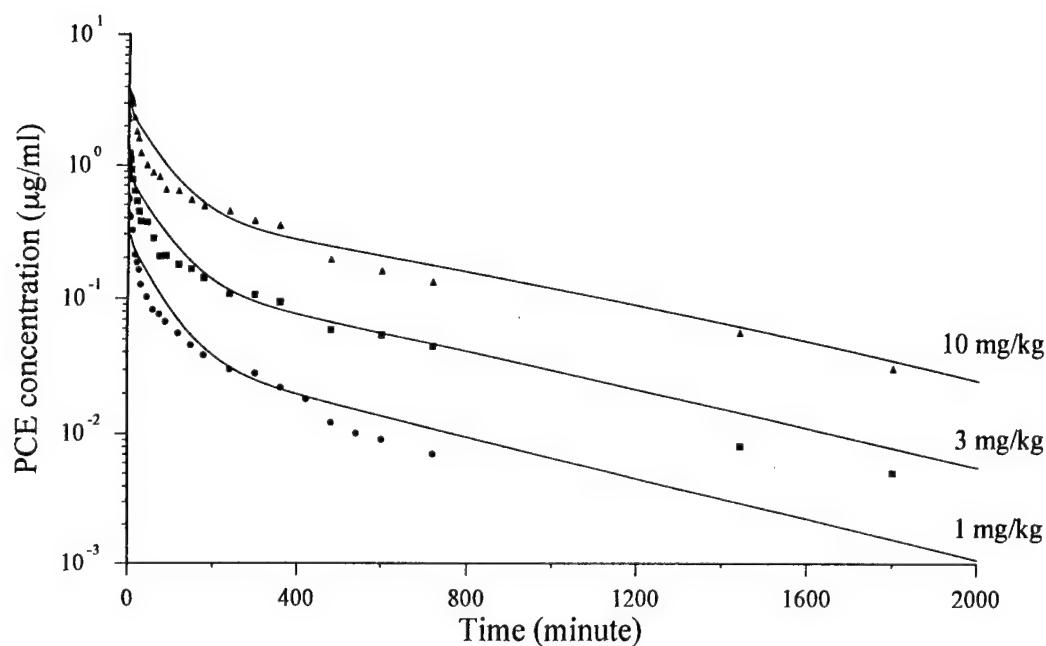


FIGURE 2. PCE concentrations in the venous blood of rats over time following i.v. administration of 1, 3, or 10 mg/kg of PCE. Each symbol represents the observed mean value for four rats, while the lines represent PBPK model-predicted values.

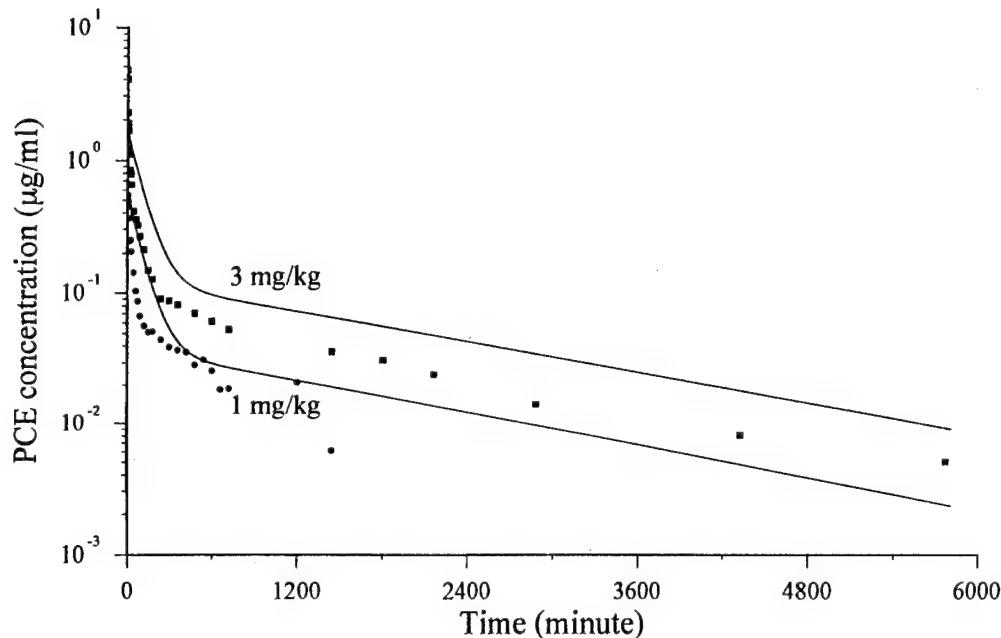


FIGURE 3. PCE concentrations in the venous blood of dogs over time following i.v. administration of 1 or 3 mg/kg of PCE. Each symbol represents the observed mean value for three dogs, while the lines represent PBPK model-predicted values.

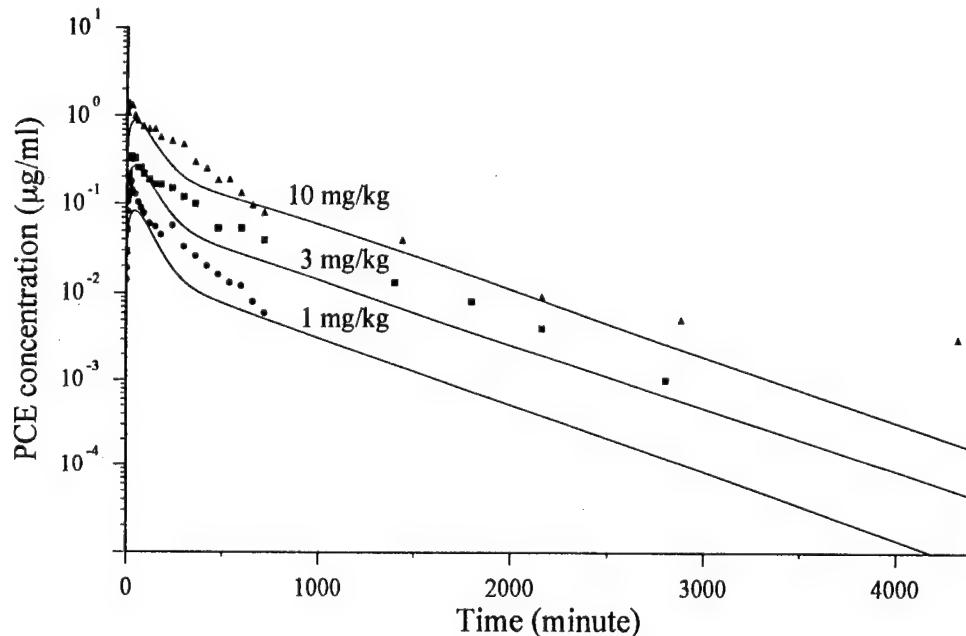


FIGURE 4. PCE concentrations in the venous blood of rats over time following po administration of 1, 3, or 10 mg/kg of PCE. Each symbol represents the observed mean value for five rats, while the lines represent PBPK model-predicted values.

concentrations were overpredicted during the initial rapid elimination phase following injection of 1 and 3 mg/kg. The difference between simulated and measured blood levels was consistent and relatively modest during the terminal elimination phase for the 3 mg/kg dose. The terminal elimination phase could not be accurately determined experimentally for the 1 mg/kg dose, as blood levels fell below the limit of detection after 20 h.

Model-generated and observed blood PCE concentrations were compared following oral administration of three doses of PCE to the rat in Figure 4. For all three doses, peak blood levels were slightly underpredicted. Simulated and observed values were in close agreement during the initial part of the rapid elimination phase. Blood concentrations during the latter portion of the rapid elimination phase and the initial part of the terminal elimination phase were somewhat underpredicted. There was generally good agreement during the remainder of the terminal phase in the 3 and 10 mg/kg groups. Unfortunately, blood PCE concentrations dropped below the limit of detection in the 1 mg/kg rats, so that the terminal elimination phase could not be defined experimentally.

Upon oral administration of PCE to the dog, model-predicted blood PCE concentrations were somewhat higher than the observed values during the rapid elimination phase (Figure 5). Blood PCE concentrations were overpredicted during the terminal elimination phase in the 10 mg/kg dogs, but well predicted in the 3 mg/kg animals.

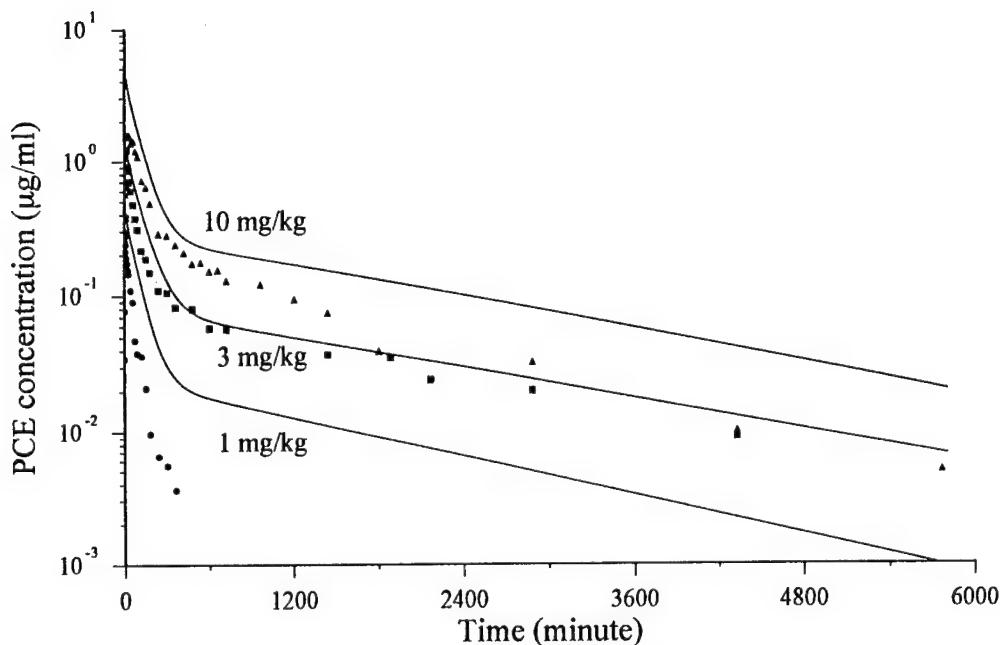


FIGURE 5. PCE concentrations in the venous blood of dogs over time following po administration of 3 or 10 mg/kg of PCE. Each symbol represents the observed mean value for three dogs, while the lines represent PBPK model-predicted values.

DISCUSSION AND CONCLUSIONS

Limited information is available on the systemic absorption of PCE from the gastrointestinal (GI) tract [Agency for Toxic Substances and Disease Registry (ATSDR), 1992]. Pegg et al. (1979) gave male Sprague-Dawley rats 1 or 500 mg ^{14}C -PCE/kg body weight in corn oil by gavage. Measurements of recovered radioactivity over 72 h following dosing indicated that absorption of both doses of PCE was virtually complete. Similar findings in mice (Schumann et al., 1980) and rats (Frantz & Watanabe, 1983) have been reported. High bioavailability (F) values were calculated for each dose of PCE given orally to rats in the current study (Table 2). Oral absorption was also extensive in the beagle dog, in that the F value for the 3 mg/kg dose was found to be about 1. Peak blood PCE levels were seen in rats 21 to 37 min postdosing. Pegg et al. (1979) did not observe peak concentrations until approximately 1 h after oral dosing. These researchers, however, employed a corn oil vehicle. Corn oil has been shown to significantly delay oral absorption of VOCs by acting as a reservoir for the lipophilic chemicals in the GI tract (Withey et al., 1983; Kim et al., 1990).

There is a paucity of blood concentration time-course data, which delineate the systemic uptake and elimination of PCE. Only one profile of blood PCE concentration versus time was found in the literature (ATSDR, 1992).

The authors took serial blood samples via a jugular cannula for up to 36 h from male Sprague-Dawley rats given 500 mg/kg of PCE orally. Limited analytical sensitivity apparently did not allow sampling at later time points, or description of the blood profile in rats receiving a 1 mg/kg oral dose. It was reported that disappearance of PCE from the blood appeared to be monophasic and followed first-order kinetics. Although they apparently did not monitor blood levels long enough to define the terminal elimination phase, their $t_{\frac{1}{2}}$ values of 7.1 to 7.4 h are comparable to the $t_{\frac{1}{2}}$ of 7.8 h for the 3 mg/kg po rats in the present study. The only other pharmacokinetic parameters estimated in this study were elimination rate constants for the blood and exhaled breath. Frantz and Watanabe (1983) reported a $t_{\frac{1}{2}}$ of 7.1 h for pulmonary elimination of PCE by male Sprague-Dawley rats ingesting about 8.1 mg/kg of the chemical in drinking water over a 12-h period.

The data sets obtained in the current investigation allow a more comprehensive assessment of the pharmacokinetics of oral PCE than has previously been possible, since the sets include detailed time-course data for three doses and two routes of administration. Increases in AUCs were somewhat more than proportional to increases in dose in rats receiving PCE orally and by ia injection (Tables 2 and 3). As would be expected, clearance diminished slightly with increase in dose in the rats. Half-life increased significantly with increase in dose in these animals. These findings are indicative of the onset of saturation of elimination processes in this dosage range (i.e., 1–10 mg/kg). It has been demonstrated previously that PCE metabolism was a saturable, dose-dependent process in male Sprague-Dawley rats, but the oral doses given (i.e., 1 and 500 mg/kg) varied markedly (Pegg et al., 1979). It has been theoretically proposed (Andersen, 1987) and demonstrated by observed measurements (Lee et al., 1991) that percent elimination of VOCs via exhalation is independent of dose. Thus, respiratory elimination of PCE and other VOCs should not be saturable. The aforementioned findings in the present study, of decreasing clearance and increasing half-life in the 1–10 mg/kg dosage range, are thus apparently the result of the onset of metabolic saturation. Male Sprague-Dawley rats that ingested 8.1 mg/kg of ^{14}C -PCE in their drinking water over a 12-h period exhaled 88% of the dose as unchanged PCE (Frantz & Watanabe, 1983). Only 7.2% and 1.7% of the dose of PCE was eliminated in the urine and feces, respectively. Thus, the rat appears to have a limited capacity to metabolize PCE. Unfortunately, it was not possible in our study to obtain data sets for the 1 mg/kg po and 10 mg/kg ia groups of dogs, and thus definitive statements about the influence of dose and exposure route on the pharmacokinetics of PCE in dogs cannot be made.

There were pronounced differences in the kinetics of ingested PCE in rats and dogs. Rats exhibited substantially lower C_{\max} and AUC values than dogs receiving the same dose of PCE by the same route. Clearance of PCE from the bloodstream was significantly slower in dogs than in rats and $t_{\frac{1}{2}}$ values were longer (Tables 2 and 3). It has been predicted that clearance should be greater in smaller species, since it increases as a fractional power of body

weight (Andersen, 1987). The major route of PCE elimination by rats (Pegg et al., 1979; Frantz & Watanabe, 1983) and by humans (Ohtsuki et al., 1983) is exhalation of the parent compound. No data apparently exist for dogs, although a PBPK model was recently used to predict significantly greater rate of PCE elimination by dogs than by rats (Dallas et al., 1991). The blood-air partition coefficient is one important factor that governs PCE exhalation. The parameter is smaller in rats (19.8) than in dogs (40.5), which favors elimination of PCE in the expired air of rats (Table 1). Two other factors that enhance respiratory elimination of PCE and other VOCs, namely, pulmonary blood flow and respiratory rate (as a function of body weight), are also markedly higher in the rat than the dog. Finally, the extent of PCE metabolism also appears to be greater in rats. It has been shown that mice metabolize a substantially larger percentage of administered doses of PCE than do rats (Schumann et al., 1980). Ohtsuki et al. (1983) demonstrated that humans have a very limited capacity of metabolize PCE. Although there are apparently no direct measurement studies of PCE metabolism by dogs, a PBPK model employed by Dallas et al. (1994) forecast significantly less metabolism of PCE by dogs than rats. Thus, it is predictable that bioavailability of PCE is greater in the larger of the two species.

The PBPK model simulation of the time-course of blood PCE concentrations in rats was in close agreement with direct measurements of the chemical for all three doses given by both routes of administration. Blood concentration over time is routinely accepted as an index of the level of chemical in the body, and therefore a representative index of certain toxic affects. Others have developed PBPK models for PCE, but the models have primarily been used to forecast exhaled breath levels and metabolite formation (Ward et al., 1988; Bois et al., 1990; Travis, 1987; Chen & Blancato, 1987). Simulations of blood and tissue concentrations of PCE over time have not been conducted, largely due to a lack of empirical data to use in parameter determination and valid action of model predictions.

The accuracy of the present model in predicting the uptake and elimination of PCE in the blood of the male Sprague-Dawley rat may be due both to the model structure and to accurate *in vivo* model input parameters. The current model is similar to models previously developed by Angelo and Pritchard (1984) and Ramsey and Anderson (1984) for other VOCs. Each model provides for inhalation and exhalation through a lung compartment, metabolism in a liver compartment, and blood-flow-limited organ representations. Our model differs from the others primarily in that it has a greater number of individual tissue compartments and thus more closely represents the anatomy of the animal. In addition, the physiological and biochemical input parameters were not taken from the literature or *in vitro* assays, but measured in the test animal (*i.e.*, the male Sprague-Dawley rat). As described in the Methods section, values measured in our laboratory in Sprague-Dawley rats were utilized for tissue volumes, cardiac output and tissue blood flows, and alveolar ventilation. *In vivo* partition coefficients were

determined in a prior investigation in which PCE was administered to rats by ia injection (Dallas et al., 1994), while metabolic rate constants were calculated from liver concentration versus time data from the same study. Hattis et al. (1990) found that there were appreciable differences in the prediction of PCE PBPK models of different researchers, which was attributed largely to variance in input parameters, notably metabolic rate constants.

The success of the PBPK model in predicting PCE blood levels in the rat provides evidence for the efficacy of using tissue-blood partition coefficients derived from in vivo tissue concentration-time data. While PBPK model development over the last decade has been greatly aided by the use of in vitro data from tissue homogenates, the disruption of normal tissue architecture and cellular structure required by the approach could result in changes in the partitioning of PCE into some tissue. For instance, in using in vitro-derived partition coefficients it was necessary in one PCE study in rats to artificially adjust the fat and muscle partition coefficients in order to sufficiently optimize the predictions relative to observed data (Ward et al., 1988). Using the parameters derived from in vivo data, then, the present model provided a more detailed and representative description of physiological structure, which was able to provide fairly accurate predictions in the rat.

The present report apparently represents the first effort to simulate the kinetics of a VOC in the dog by use of a PBPK model. The model generally overpredicted blood PCE concentrations somewhat following po and ia administration of the halocarbon. This difference from measured blood levels was very likely due to the inaccuracy of certain input parameters, rather than incorrect model structure. As for the rat, it was possible to accurately determine some in vivo parameters from the dog blood concentration time-course data (i.e., blood-air and tissue-blood partition coefficients and metabolism constants). It was necessary to obtain other input parameters from the literature. Citations were found that spanned many years, a variety of techniques, and different types of dogs. Where possible, values were taken from publications employing state-of-the-art techniques (e.g., measurement of tissue blood flows by a radiolabeled microsphere technique) in unanesthetized beagles. Where more than one investigator employed the same technique in beagles, the median value was used. Nevertheless, there was uncertainty about the validity of the literature values which were selected. Measurement and utilization of precisely determined physiological parameters in the dog should improve the accuracy of PBPK model predictions of the disposition of PCE and other VOCs in that species.

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APPENDIX G

You, L., Muralidhara, S., and Dallas, C.E. "Comparisons between operant responding and 1,1,1-trichloroethane toxicokinetics in mouse blood and brain." *Toxicology*, 93: 151-163 (1994).



Comparisons between operant response and 1,1,1-trichloroethane toxicokinetics in mouse blood and brain

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Abstract

The effect of 1,1,1-trichloroethane (TRI) inhalation on operant response was evaluated in relation to the concentration of TRI in blood and brain tissue in mice during exposure. Male CD-1 mice were trained to lever-press for an evaporated milk reinforcer on a variable interval (VI 60) schedule for 2 h. Trained mice were then exposed to either 3500 or 5000 ppm TRI for 100 min, and the changes in the schedule-controlled performance were measured. Additional groups of mice were exposed under the same conditions as those used in the behavioral study and sacrificed at various times during exposure, and the blood and brain samples were collected and subsequently analyzed for TRI content by headspace gas chromatography. Uptake of TRI into blood and brain was rapid, with near steady-state levels reached after approximately 40–60 min of exposure. Inhalation of 5000 ppm, but not 3500 ppm TRI was seen to cause inhibition of operant response, starting ~30 min following the initiation of inhalation exposure and beginning to recover after 80 min of exposure. The threshold concentrations for the maximal behavioral inhibition were ~110 µg/g and 130 µg/ml in mouse brain and blood, respectively. It appears that in addition to TRI concentrations in blood and brain tissue, the time it takes to reach the apparent threshold TRI concentration was also a determinant for the onset of TRI neurobehavioral depression.

Keywords: 1,1,1-Trichloroethane; Operant responding; Neurobehavioral depression; Toxicokinetics

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1. Introduction

1,1,1-Trichloroethane (TRI), also known as methyl chloroform, is a chlorinated aliphatic hydrocarbon used as a solvent in a large number of industrial and commercial products. The world production of TRI was ~680 000 tons in 1988, of which ~50% was produced in the US. (Arlien-Søborg, 1992; Dobson and Jensen, 1992). TRI is considered to have a relatively low degree of toxicity, with central nervous system (CNS) depression as the reported primary effect after high levels of exposure in humans (Kleinfeld and Feiner, 1966; Stewart, 1968; Torkelson and Rowe, 1981). Following animal studies of very high doses of TRI, cardiac arrhythmias (Reinhardt et al., 1973; Herd et al., 1974) and hepatic and renal toxicity (Plaa and Larson, 1965; Klaassen and Plaa, 1966, 1967) have also been reported.

Because of their high volatility, inhalation is the primary route of exposure to most volatile organic compounds (VOCs). Inhalation exposure to TRI occurs frequently in occupational settings as well as through intentional intoxication with commercial products. Previous studies have shown that TRI is able to affect CNS functions in several experimental models, and its toxicity has been expressed as a disturbance of neurobehavioral functions similar to that of depressant drugs (Rees et al., 1987; Evans and Balster, 1991, 1993). It is important to assess changes in CNS basal activity in a valid quantitative manner (Horwath and Frantik, 1973; Filov et al., 1979). Repetitive, on-line determinations of neurobehavioral response concurrent with solvent exposure are of significant utility in elucidation of the time course of CNS effects of these compounds (Balster et al., 1982). Operant performance measurements have been shown to be useful in the detection of subtle CNS effects of low VOC exposure concentrations prior to reaching a level which would result in irreversible neuropathological changes (Geller et al., 1979). CNS-depressant effects of VOCs have been demonstrated in animals by operant tests at doses (Wood et al., 1983) similar to those that have been shown to alter human performance (Gamberale and Hultengren, 1972). TRI has been found in several animal models to reduce response rates in operant behavior of different reinforcing schedules (Balster et al., 1982; Geller et al., 1982; De Ceaurriz et al., 1983).

It has been shown in the case of toluene that the magnitude of the solvent-induced performance inhibition was correlated with the concentration of parent compound in the CNS of mice (Bruckner and Peterson, 1981). Although the highest degree of correlation was consistently seen between the extent of CNS depression and brain toluene concentration, blood levels were also a reasonably reliable index of the depth of narcosis. However, there is a general paucity of data for most VOCs that links toxicokinetics of solvents and their neurobehavioral effects. The purpose of this study therefore, was to demonstrate the effects of TRI on variable-interval response in mice and examine the relationship between the neurobehavioral effect and the chemical concentration in the blood and brain tissue.

2. Methods

2.1. Animals

CD-1 male mice from Charles River Breeding Laboratories (Raleigh, NC),

weighing 30–35 g, were used in the study. The mice were housed on a constant light-dark cycle, with light from 07:00–19:00 h, and darkness from 19:00–07:00 h, in standard mouse cages. The mice were acclimated to the animal facility for at least 1 week, during which tap water and commercial rodent chow were provided ad libitum. The mice were then housed individually and restricted in food intake so that their body weight would be reduced over 1 week to and maintained at 80% of the original weight. Water access was unlimited. In the kinetic study, the mice were fed in the same way as in the behavioral study for 3 weeks. The mice were maintained at this weight until used in the kinetic and behavioral studies.

2.2. Chemicals

1,1,1-Trichloroethane, of 99% purity and iso-octane, of 99.98% purity, were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ).

2.3. Inhalation exposures

Inhalation exposures were conducted in 1.0 m³ Rochester-type dynamic flow chambers. Test atmospheres of TRI were generated by vaporization of the compound under different combinations of air flow- and temperature-controlled schedules. A flow of nitrogen gas carried the vaporized TRI from a flask into the exposure chamber influent air stream. The entire TRI generation system was enclosed in a specially fabricated safety box, which was maintained under constant negative pressure during inhalation exposures in the same manner as the inhalation chamber. Exhaust air from the vapor-generation box and the inhalation chamber were vented to HEPA and charcoal filters, so that all air with any chemical was scrubbed before release to the environment. The exposure chamber was operated at flow rates of 0.2–0.42 m³/min. The concentration of TRI in the exposure chamber was monitored by an on-line Miran 1B2 IR spectrophotometer (Foxboro Analytical, South Norwalk, CT) on the level of the bottom of the operant cage. Clean air was pumped through the chamber following the termination of the inhalation exposure.

In the behavioral study, modular operant cages for mice (Coulbourn Instruments, Allentown, PA) were positioned inside the inhalation chamber throughout the training and exposure sessions. The operant cages had been adapted so that TRI vapors could move freely through the cages during operant sessions. In the kinetic study, mice were exposed to TRI at the same inhalation exposure concentrations as in the behavioral study.

2.4. Operant study

With slight modifications, operant testing in the present study was conducted according to the method described by Balster et al. (1982). Briefly, the mice were food restricted during the period in which they were shaped to lever-press for 0.01 ml evaporated milk presentation, and trained to a variable-interval 60 s (VI 60) schedule in 2-h daily sessions in the modular operant cages. The baseline response rates were determined from operant sessions spaced 24 h apart. Output of the operant cage was analyzed by a modular behavioral analysis instrument panel (Coulbourn Instruments, Allentown, PA). The schedule contingencies and data recording were carried out by a computer program, COSMOS (Coulbourn Instruments, Allentown,

PA), that was run on an IBM-compatible 386 computer. The number of lever presses in each 5-min period of the operant session was recorded for both baseline and exposure sessions. A stable performance was judged by two criteria: (i) the standard errors of the operant response rates in each of those three behavioral sessions immediately before the inhalation exposure fell within 10% of their means; (ii) the variations in means of each session became less than 10% of their grand mean. After the schedule-controlled response became stable, the mice were exposed to clean air for 20 min followed by either 3500 or 5000 ppm TRI for 100 min. Response ratios were calculated by dividing the number of responses during exposure by the average number of responses during the three control sessions immediately preceding exposure.

2.5. Kinetic study

The prepared mice were exposed to either 3500 or 5000 ppm under the same conditions as the behavioral study for either 10, 20, 40, 60, 80, or 100 min at which time blood samples (0.5 ml) were withdrawn by closed chest cardiac puncture immediately after the mice were sacrificed by cervical dislocation. Samples of brain were quickly removed and were immediately placed into ice-chilled scintillation vials containing 4 ml of iso-octane and 1 ml of 0.9% NaCl and tightly capped. The tissues were homogenized using the Ultra-Turrax SDT homogenizer (Tekmar, Cincinnati, OH) for 5 s. The vials were then vortex-mixed for 30 s and centrifuged at 1800 × g for 10 min at 4°C in capped vials. Aliquots of the iso-octane supernatant layer were taken into 20-ml vials, which were capped immediately with teflon-lined rubber septa and crimped to insure an air-tight seal. The capped vials were then placed into a headspace auto-sampler unit of a Perkin-Elmer Model 8500 gas chromatograph (GC) (Perkin-Elmer, Norwalk, CT). Analyses were carried out using a stainless-steel column (182 cm × 0.317 cm) packed with 3% OV-17 (Alltech Associates, Deerfield, IL). The GC operating conditions were headspace sampler temperature, 70°C; injection port temperature, 150°C; column temperature, 80°C; detector temperature, 360°C. Each sample vial was heated thermostatically for 30 min in the GC autosampler unit, pressurized for 30 s with the carrier gas, and a fixed volume of the iso-octane supernatant injected automatically into the GC column. The limit of detection for TRI by GC was less than 1 ng in 20 ml air, or 8.4 ppb TRI in the sample vial. The recovery rates of TRI in blood and tissue from the procedure were determined prior to the kinetic study using the method described by Chen et al. (1993). Briefly, samples of blood and brain tissue were taken from chemical-naive mice ($n = 10$) immediately after they were terminated by cervical dislocation. Each sample was spiked with TRI by injection of 4 µl of a solution containing 1 mg/ml TRI in iso-octane. All of the spiked samples were then processed as described above. The % recovery was derived by dividing the amount of TRI resulting from the GC analyses by 4 µg, the amount injected into the samples.

Animal training was arranged between 09:00–17:00 h, 7 days per week. Each animal was trained in the same period of time each day. The training and exposure sessions were carried out at a same time period throughout the study. All the inhalation exposures for the kinetic study were conducted between 09:00–12:00 h. The collected samples were processed and analyzed immediately following exposure.

2.6. Statistics

A repeated measures analysis of variance was employed to determine the difference between the pre-TRI response rates and the response rates during the treatment sessions as well as to examine the effects overtime. An independent *t*-test was used to detect differences in TRI tissue concentration of 3500 ppm and 5000 ppm groups and between the baseline operant response rates of the two groups. The level of significance was set at $P < 0.05$ in all tests.

3. Results

It took less than 3 min for TRI concentrations in the inhalation exposure chamber to reach the target concentrations of 3500 and 5000 ppm TRI after the initiation of exposure. TRI concentrations were maintained at the level of the target concentration $\pm 5\%$. The values ($n = 10$) of % recovery of TRI from the spiked blood and brain tissue samples were 94.7 ± 2.4 (mean \pm S.E.) and 90.5 ± 1.9 for blood and brain, respectively. These values were subsequently factored into the calculation of TRI concentrations.

In the behavioral study, all animals that were subsequently tested had achieved a consistent performance that was maintained for at least 3 days before the TRI exposures were conducted. The standard errors of the baseline operant response rates within each operant session ranged from 3.4% to 9.6% of their means. The baseline performance was also maintained during the initial 20 min of the clean air exposure before the chemical was introduced. The baseline response rates across animals in each group of mice were 153 ± 13.6 and 142 ± 14.4 (Mean \pm S.E.) for each 5-min period in the 3500 and 5000 ppm groups, respectively. An independent *t*-test revealed no statistically significant difference between the baseline response rates for the two groups of mice. The repeated measures analysis of variance was applied to both dose levels. At 3500 ppm level inhalation exposure, the mice tested ($n = 7$) showed no statistically significant decrease in operant response rates comparing to the baseline values throughout the 100 min exposure session, although there appeared to be a small attenuation in response rate following the introduction of TRI. The response rates stayed at $\sim 85\%$ of the base rate after the initiation of exposure with the highest at 88% and the lowest of 81% (Fig. 1a). At 5000 ppm level, the mice ($n = 5$) showed a trend of reduced response rates starting at ~ 25 min of inhalation exposure, although this reduction was not statistically significant until after 45 min of exposure. This depression of response lasted ~ 40 min (except at 50 min), and afterward the depression gradually recovered and the operant response rates returned to baseline level (Fig. 1b). It was also revealed that changes in response rates over time were significant and there was an interaction between treatment and exposure time in the 5000 ppm group.

The uptake of TRI into blood and brain tissues was rapid, and the concentrations in both blood and brain increased steadily over time (Fig. 2a,b). Concentrations in blood samples reached near steady-state within 40 min of inhalation exposure, whereas it took ~ 60 min in the brain tissue. The highest TRI concentrations measured at 100 min in blood were 141 and 198 $\mu\text{g}/\text{ml}$ for 3500 and 5000 ppm levels, respectively; while the highest concentrations (also at 100 min) measured in brain

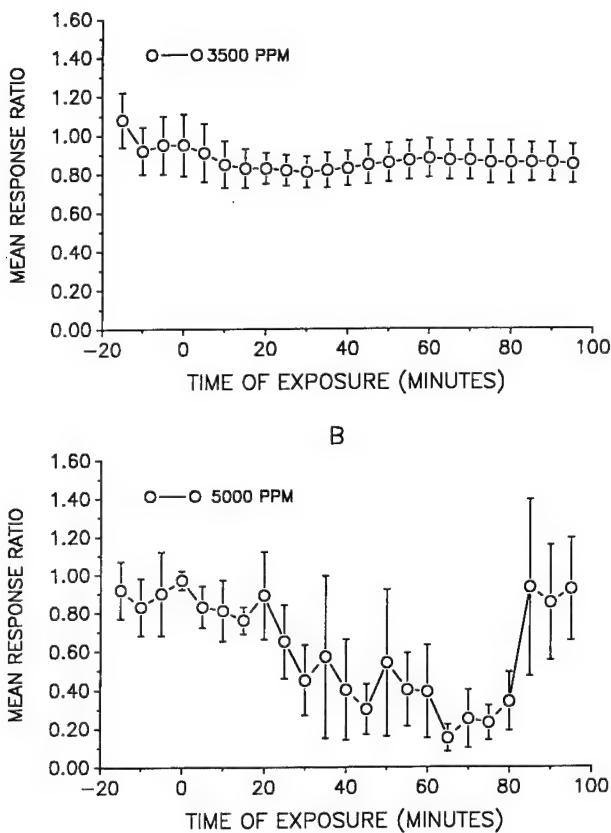


Fig. 1. Mean response ratios (\pm S.E.) for a 20-min exposure to clean air following by 100 min of exposure to (a) 3500 ppm or (b) 5000 ppm TRI. Mice were trained to perform under a variable-interval 60 s schedule of milk presentation until attaining a stable response.

were 134 and 189 $\mu\text{g/g}$ tissue. In blood, TRI concentrations of the 3500 and 5000 ppm groups were significantly different at 60, 80 and 100 min. There were significant differences in brain concentrations between the two exposure groups at 40, 60, 80 and 100 min.

The TRI brain concentration was 114 $\mu\text{g/g}$ at 40 min in the 5000 ppm group, when the operant response ratio showed a significant decrease relative to baseline response. However, when the TRI brain concentration in the 3500 ppm group reached approximately the same level at 80 min (113 $\mu\text{g/g}$) and surpassed it at 100 min with 134 $\mu\text{g/g}$, there was no significant decrease in the operant response rate. Also, when the depressed operant response rate in the 5000 ppm group returned to baseline level after chemical exposure for 80 min, the brain and blood TRI concentrations continued to increase.

Pooling the data for both inhalation concentration exposure groups, the mean response rates of the operant tests were plotted against brain (Fig. 3a) and blood (Fig.

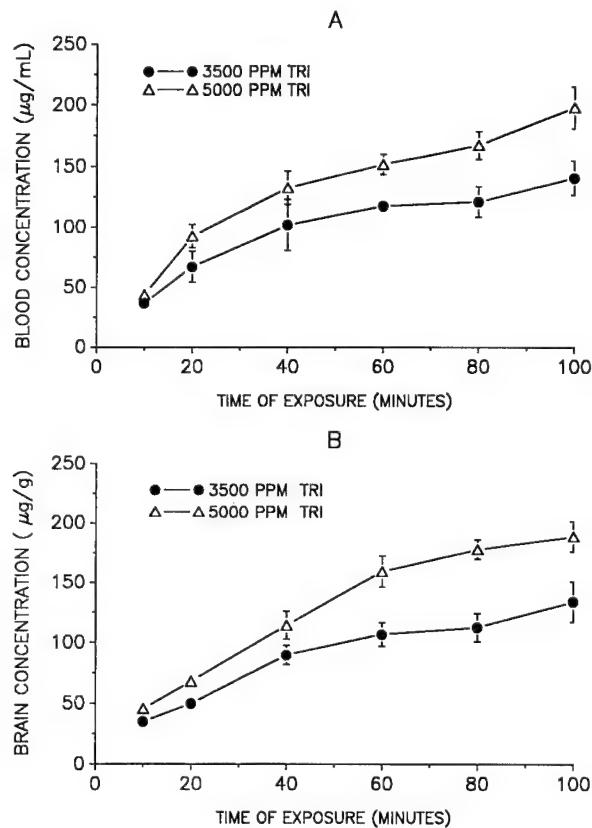


Fig. 2. Concentrations of TRI in the (a) blood and (b) brain of mice following exposure to either 3500 ppm or 5000 ppm TRI for up to 100 min. Serial sacrifices were conducted at 10, 20, 40, 60, 80 and 100 min and TRI analyzed by headspace GC. Values are expressed as the mean \pm S.E. of 5 mice at each point.
*Statistically significant difference with 3500 ppm group, $P < 0.05$.

3b) TRI concentrations. For the blood data, mean response ratios remained between 80–90% until 120 $\mu\text{g}/\text{ml}$ TRI was achieved after which there was a general decline. The same was true of mean response ratios when mean brain concentrations were below 110 $\mu\text{g}/\text{g}$. Above 150 $\mu\text{g}/\text{ml}$ concentrations in blood and 160 $\mu\text{g}/\text{g}$ in brain the mean response ratios plummeted to 30–40% of control levels. However, in both cases, data points were distributed as two clusters, and the second degree regression models only displayed a moderate fit.

4. Discussion

The toxicokinetic relationship between the uptake and disposition of chemicals and their toxic effects has been recognized as an increasingly important factor in risk assessment (Clark and Smith, 1984; Clewell and Andersen, 1985). Among the studies

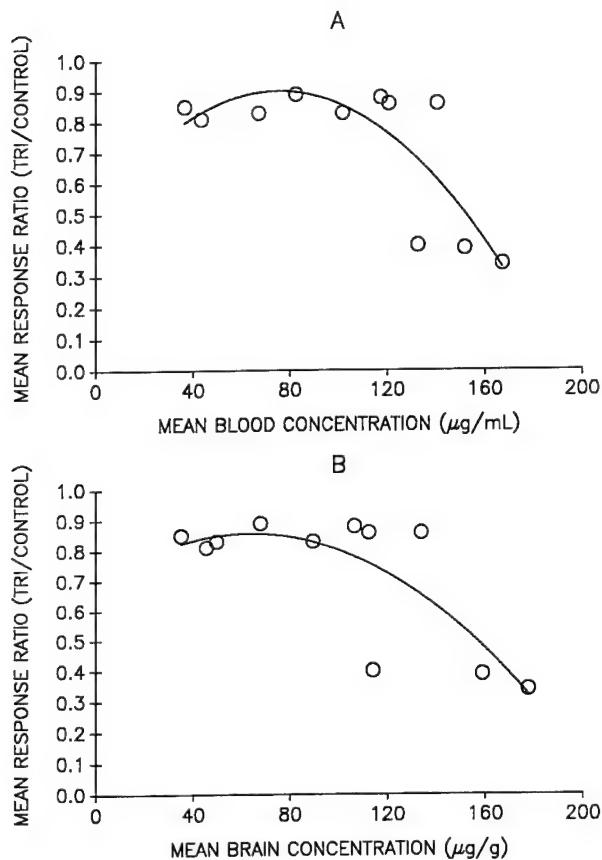


Fig. 3. The relationship between operant response rates and (a) blood TRI concentrations, and (b) brain TRI concentrations. The values were pooled from both the 3500 and 5000 ppm groups. Curves for the relationship are generated by a second order regression. The equation for the blood and mean response ratio is $y = 0.514 + 0.01x - 6.81 \cdot 10^{-5} x^2$ and $r = 0.799$; and the equation for the brain and mean response ratio is $y = 0.689 + 5.25x \cdot 10^{-3} - 4.12 \cdot 10^{-5} x^2$ and the $r = 0.775$.

conducted on the pharmacokinetics of VOCs, there has been a particular paucity of data obtained during inhalation exposure for most VOCs. Likewise, there have been few studies that measured blood and tissue TRI concentrations during inhalation exposure. Uptake of TRI into the arterial blood of rats during inhalation exposure was found to be rapid, with a near steady-state equilibrium achieved after ~30–40 min (Dallas et al., 1991). In the current study with mice, blood TRI levels also reached near steady-state ~40 min into exposure at both dose levels, whereas TRI brain concentrations achieved near steady-state within 60 min of exposure. This rapid equilibration demonstrates the importance of the characteristic volatility of TRI and other VOCs in determining the rate of uptake following inhalation exposure. Using a comparison for which data are available, this rapid uptake of TRI in the current study

for mice and previously in rats (Dallas et al., 1989) is considerably faster than that of toluene, which reached steady blood and brain concentrations only after >2 h of inhalation exposure at 4000 ppm level (Bruckner and Peterson, 1981). The difference in pharmacokinetics of the two chemicals can be largely attributed to differences in their partition coefficients, and particularly the blood:air partition coefficient, which is 15.6 for toluene (Sato and Nakajima, 1979a) and 3.3 for TRI (Sato and Nakajima, 1979b). It was shown by Reitz et al. (1988) that after inhalation exposure to TRI at 150 and 1500 ppm levels for 6 h, mice blood concentrations of the chemical were 9.27 and 111 µg/ml, respectively. A study conducted by Schumann et al. (1982) demonstrated similar results of TRI blood concentrations in mice with 12.6 and 105.3 µg/g for 150 and 1500 ppm, respectively, following inhalation exposure for 6 h. If one assumes a linear scale-up to the 3500 and 5000 ppm exposures used in the current study, the blood levels would be 233 and 333 µg/ml, respectively at the end of the 6 h exposure. These values would be 66% and 68% higher than the levels measured at 100 min in the present study, as we detected 141 and 198 µg/ml of blood TRI at 100 min exposure for 3500 ppm and 5000 ppm, respectively. Because of saturation kinetics in the current study after 40–60 min exposure, the aforementioned difference cannot be completely attributed to longer exposures in previous studies. Therefore, a simultaneous scale-up on dimensions of both exposure period and dose seems inappropriate. It was found, however that the kinetics of TRI in mice was linear over the high doses employed. TRI concentrations in the blood and brain after 100 min of exposure both increased 1.41-fold, with a 1.43-fold increase in the inhalation exposure concentration (from 3500 to 5000 ppm). Similar findings have been found following TRI inhalation exposures in mice and rats (Schumann et al., 1982; Dallas et al., 1991), and in humans (Nolan et al., 1984). It was reported in these studies that concentrations in blood and tissues, exhaled breath, and body burden of TRI were each proportional to dose in these species.

The effects of TRI on the CNS, as determined by measurements of schedule-controlled behavior, have been demonstrated by several studies (Balster et al., 1982; Moser et al., 1985; Warren et al., 1993). Using different exposure protocols, operant schedules, and two species (mice and rats), the experimental animals generally showed depression in their operant response within minutes of the initiation of exposure to concentrations higher than 2000 ppm. In the current study this inhibition effect was not observed in the 3500 ppm group, and at 5000 ppm it was not until 45 min into the exposure that the operant response began to be significantly depressed. All of the previous studies in mice used a fixed-ratio performance schedule, which probably accounts for differences in the time of onset of CNS depression to TRI inhalation as well as different sensitivity to exposure concentration. It is known that the use of different schedules to which operant response is reinforced often cause different sensitivities to toxicological insult (Laties and Wood, 1986; Cory-Slechta, 1992). These differential effects of reinforcement schedules have been shown for fixed-ratio and variable-interval as well as other types of reinforcement (Davey, 1981). While the underlying mechanism has been subject to various behavioral interpretations, the rates and patterns of operant response are thought to be among the primary determinants of the ability of an agent to enhance or inhibit the established

operant behavior (Davey, 1981; Glowa, 1990). It has been shown that CNS depressants like pentobarbital and some other VOCs (e.g. toluene) can cause rate-dependent effects on operant behavior using different schedules. Because a lower rate of response is associated with variable-interval relative to fixed-ratio schedule, the former may be less sensitive to the neurobehavioral effects of TRI in mice.

An interesting toxicokinetic finding is that there was recovery from the CNS behavioral inhibition during the latter part of the 100-min exposure to 5000 ppm TRI, despite the continued near steady-state blood and brain concentrations of TRI during the period. At 80 min of exposure, where recovery started, the brain and blood concentrations of TRI were 178 µg/g and 167 µg/ml, respectively, and there was a slight trend to increasing concentration. Because there have not been any reported studies in mice that have had operant sessions concurrent with TRI exposure lasting as long as 80 min, comparison with other studies in this respect are not available. For example, Balster et al. (1982) monitored operant behavior during 20 min of exposure to TRI. It might be feasible that there was a development of acute tolerance as an adaptive change during exposure, due to exposure length and operant schedule employed. When a behavioral phenomenon has been the end point of evaluation, it is known that tolerance may develop more readily when the effect of the agent has a behavioral 'cost' to experimental animals such as when it reduces the capability to obtain a reward or to avoid punishment (Jaffe, 1990). In order to achieve a more complete understanding of behavior as a function of CNS processes, behavioral endpoints need to be co-ordinated with physiological and biochemical variables, of which pharmacokinetics is a particularly valuable candidate (Weiss, 1988). Most co-ordinated assessments of behavior and pharmacokinetics have had to overcome formidable technical limitations. For example, Gallaher et al. (1982) had to assay ethanol in blood samples taken from a snipped tail vein of mice for a single time point for comparison with ataxic effects of ethanol. Geller et al. (1982) pointed out the difficulties inherent in measuring blood and tissue levels of the inhaled compound in conjunction with operant sessions, and had to make assumptions of the pharmacokinetic behavior of TRI in the absence of data to co-ordinate with their neurobehavioral measurements. Blood and brain levels of toluene were compared to the neurobehavioral effects of toluene during and following inhalation exposure (Bruckner and Peterson, 1981). Toxicity was measured in a battery of tests of unconditioned performance and reflex to assess CNS effects in mice and rats. There was a good correlation between degree of behavioral depression and both blood and brain concentrations during and following inhalation exposure. In the current study, blood and brain concentrations of TRI were related to behavioral depression, but were certainly not the only factors that dictated the resulting behavioral effect over time. The length of time that it took to reach certain brain concentrations associated with behavioral depression seemed to also be an important factor. The brain TRI concentration at 5000 ppm dose level was 114 µg/g at 40 min when a significant inhibition of operant response was observed. Brain concentrations in the mouse inhaling 3500 ppm reached a similar level at 80 min (113 µg/g) and exceeded it at 100 min with 134 µg/g. It might be postulated that the use of the variable-interval schedule only allowed the detection of CNS depression at the

higher dose, since the TRI concentration necessary to elicit a response was achieved considerably earlier in the operant session at the higher dose. Indeed, when time is eliminated in pooled comparison of blood or brain TRI concentrations and behavioral deficit (Fig. 3), only a moderate correlation is available from second degree regression curves. Because of the possibility of the effect of time of exposure on the onset of behavioral depression, singular use of blood or brain concentration to determine the behavioral toxicity of TRI inhalation in mice may result in inaccurate predictions.

Acknowledgements

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APPENDIX H

You, L., Muralidhara, S., and Dallas, C.E. "The pharmacokinetics of inhaled 1,1,1-trichloroethane following high milk intake in mice." *Drug and Chemical Toxicology*, 17: 479-498 (1994).

**THE PHARMACOKINETICS OF INHALED 1,1,1-TRICHLOROETHANE
FOLLOWING HIGH MILK INTAKE IN MICE**

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ABSTRACT

In the evaluation of lipophilic halocarbons for neurobehavioral toxicity in operant testing, animals often receive large amounts of milk as a behavioral reinforcer over time. If this increase of fat in the diet sufficiently impacted the lipid depots of the animal, the pharmacokinetics of lipophilic test compounds might be significantly affected and thus obscure the accompanying neurobehavioral effects. The effects of milk intake, comparable to what was consumed as behavioral reinforcer during operant behavioral sessions, on the pharmacokinetics of inhaled 1,1,1-trichloroethane (T1) were therefore examined in the blood and nine organ tissues of mice. Male CD-1 mice were food restricted

so that their body weights would be reduced to and maintained at 80% of their original, and received a single gavage dose of 1.0 ml evaporated milk daily for three weeks. A control group with similar food restrictions was dosed with the same volume of water. Inhalation exposures to 3500 ppm TRI for 100 minutes were conducted at the end of the treatment period. Blood and nine organ tissues were sampled at a series of time points, and their TRI contents were analyzed by headspace gas chromatography. The uptake of TRI was rapid, with near steady state approached in blood and most tissues after 40-60 minutes of exposure. All of the tissues except fat had similar TRI time-concentration profiles, while TRI concentrations in fat tissue were about 20-30 times higher than in other tissues. There was no statistically significant difference in the tissue concentrations between the milk-dosed group and water-dosed group at all of the time points for all tissues measured. Therefore, it appears unlikely that this level of milk intake as a reinforcer in behavioral studies will affect the results of operant testing evaluations by altering the pharmacokinetics of lipophilic halocarbons such as TRI.

INTRODUCTION

1,1,1-Trichloroethane (TRI) is a volatile organic compound (VOC) widely used in many industrial and commercial products. Regarded as of relatively low toxicity, TRI has nonetheless been shown to be associated with some adverse health effects in animals as well as in humans when administered at high dose

levels¹. Because inhalation is the primary route of exposure for many VOCs in both occupational settings and intentional intoxication with commercial products, it is frequently employed in investigations of TRI toxicity. Previous studies have shown that, in addition to cardiac arrhythmias^{2,3} and hepatic and renal toxicity⁴⁻⁶, the acute toxicity of TRI at high doses is most often characterized by central nervous system (CNS) depression⁷⁻⁹.

In the studies on the CNS effects of VOCs, schedule-controlled behavior has been frequently employed as a quantitative endpoint to measure neurobehavioral toxicity^{10,11}. The inhalation of TRI by test animals has been associated with reductions in the responding rates of operant behavior using various reinforcing schedules¹²⁻¹⁴. Frequently, milk presentation has been used as a reinforcer to establish the operant behavior in those studies on solvent neurobehavioral toxicity¹⁵⁻¹⁹. Depending on the animal model and experimental protocol of the study, shaping animals to perform reliably on a reinforcing schedule to be used in operant measurements has usually been a lengthy process (often taking weeks), and the amount of the milk consumed by the animals over the period of the study may be considerable.

It has been recognized that diet is one of the factors that can alter the pharmacokinetics and toxicity of a compound. The pattern, quantity, and content of dietary intake is able to change the responsiveness of a biological system to a toxicant, as well as to influence the bioavailability of the chemical in the system^{20,21}. In the case of VOCs, it has been demonstrated that pharmacokinetics are directly correlated with the subsequent neurobehavioral toxicity for TRI¹⁵ and

toluene²². However, the consequence of high milk intake on VOC pharmacokinetics that would occur during the behavioral sessions of neurobehavioral toxicity studies has not been examined previously. Since an effect on the pharmacokinetics of a toxicant often results in an alteration in toxicity, this study was undertaken to determine the potential impact of high milk intake during operant training on the pharmacokinetics of inhaled VOCs.

METHODS

Animals

CD-1 male mice from Charles River Breeding Laboratories (Raleigh, NC), weighing 30-35 g, were used in the study. The mice were housed on a constant light-dark cycle, with light from 0700 to 1900 hr, and darkness from 1900 to 0700 hr, in standard mouse cages and acclimated to the animal facility for at least one week, during which tap water and commercial rodent chow were provided ad libitum. The mice were then housed individually and restricted in food intake so that their body weight would be reduced over one week to and maintained at 80% of the original weight. Water access was not limited. Starting with the food restriction, the mice were administered a single bolus dose (1.0 ml) of evaporated milk, which has a fat content of 85 mg/ml, by gavage on a daily basis. A control group with similar food restrictions was gavaged with same volume of water. The body weight of the animals was recorded daily, and the amount of the food (Mouse Chow 5010) given to each mouse was adjusted to maintain their body

weight at 80% of the original stable weight of the animals. This was done between 1100 to 1200 hr every day, seven days a week, and the mice were maintained at this feeding protocol for three weeks until the chemical exposure.

Chemicals

1,1,1-Trichloroethane, of 99% purity was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ).

Inhalation Exposures

Inhalation exposures were conducted in 1.0 M³ Rochester-type dynamic flow chambers. Test atmospheres of TRI were generated by vaporization of the compound under different combinations of air flow- and temperature-controlled schedules. A steady flow of nitrogen gas carried the vaporized TRI from a flask into the exposure chamber influent air stream. The entire TRI generation system was enclosed in a specially fabricated safety box, which was maintained under constant negative pressure during inhalation exposures in the same manner as the inhalation chamber. Exhaust air from the vapor-generation box and the inhalation chamber were vented to HEPA and charcoal filters, so that all air with any chemical was scrubbed before release to the environment. The exposure chamber was operated at flow rates of 7 to 15 ft³ per minute. The concentration of TRI in the exposure chamber was monitored by an on-line Miran 1B2 infrared spectrophotometer (Foxboro Analytical, South Norwalk, CT) on the level of the mouse cage. Clean air was pumped through the chamber following the termination of the inhalation exposure.

Determination of Percentage of Recovery

The recovery rates of TRI in blood and tissues from the sample processing procedure were determined prior to the kinetic study, using the method described previously for rats by Chen et al.²³. Ten chemical-naive mice were terminated by cervical dislocation. Blood samples of 0.5 ml were immediately withdrawn by closed chest cardiac puncture. The whole organ of spleen and portions (0.2-0.4 g) of liver, kidney, fat, muscle, heart, lung, gastro-intestinal tract (GI), and brain were removed and placed on an ice-chilled glass plate. Each sample was spiked with TRI by injection of 4 μ l of a solution containing 1 mg TRI per 1 ml isoctane. All of the spiked samples were then processed immediately, and their TRI contents were analyzed as described in the following section.

Kinetic Study

The mice that had been food restricted and gavaged with either milk or water for three weeks were exposed to 3500 ppm for either 10, 20, 40, 60, 80, or 100 minutes. At each time point blood samples (0.5 ml) were withdrawn by closed chest cardiac puncture immediately after the mice were sacrificed by cervical dislocation. Samples of liver, kidney, fat, muscle, heart, lung, gastro-intestinal tract (GI), spleen and brain were quickly removed and were immediately placed into ice-chilled scintillation vials containing 4 ml of isoctane (99.98% purity) and 1 ml of 0.9% NaCl and tightly capped. The tissues were homogenized using a Ultra-Turrax SDT homogenizer (Tekmar, Cincinnati, OH) for 5-15 seconds. The vials were then vortex-mixed for 30 seconds and centrifuged at 1800 x g for 10 min at 4°C in capped vials. Aliquots of the

isooctane supernatant layer were transferred into 20 ml headspace vials, which were capped immediately with teflon lined rubber septa and crimped to insure an airtight seal. The capped vials were then placed into a headspace autosampler unit of a Perkin Elmer Model 8500 gas chromatograph (GC) (Perkin-Elmer, Norwalk, CT). Analyses were carried out using a stainless-steel column (182 cm x 0.317 cm) packed with 3% OV-17 (Alltech Associates, Deerfield, IL). The GC operating conditions were headspace sampler temperature, 70°C; injection port temperature, 150°C; column temperature, 80°C; detector temperature, 360°C. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 sec with the carrier gas, and a fixed volume of the isooctane supernatant injected automatically into the GC column. The limit of detection for TRI by the GC was less than 1 ng in 20 ml, or 8.4 ppb TRI in the air of the sample vial. All of the inhalation exposures for the kinetic study were conducted between 0900 to 1200. The collected samples were processed and analyzed immediately following the exposure.

An independent t-test was used to detect the difference in TRI tissue concentrations between the milk-dosed and water-dosed groups. The level of significance was set at $p < 0.05$.

RESULTS

The reductions of body weights to 80% of their originals for all of the animals were achieved within one week following the start of food restriction, and

TABLE 1

Recovery of 1,1,1-Trichloroethane from
Mouse Blood and Tissues of Different Organs

Samples	Recovery (%)*
Liver	92.5 ± 1.7
Kidney	91.8 ± 1.8
Fat	88.1 ± 2.1
Muscle	92.4 ± 1.2
Heart	90.6 ± 1.0
Lung	95.5 ± 1.4
GI	91.8 ± 2.4
Spleen	86.6 ± 1.1
Brain	90.5 ± 1.9
Blood	94.7 ± 2.4

*Values represent the mean ± SE for recovery of TRI from blood and tissues of ten mice.

the average body weight afterward stayed within ±5% of the target for all animals.

The mean values (N=10) of % recovery of TRI from the spiked blood and tissue samples are presented in Table 1. The lowest recovery rate (86.6%) was found in spleen, which had the smallest tissue mass (about 0.1 g) available for the processing procedure. The highest recovery (95.5%) was from lung tissue, which was followed closely by recovery from blood (94.7%). Indeed, all tissues but spleen and fat had greater than 90% recovery. The ten tissues showed an average of 91.45 ± 2.7% (mean ± SD) recovery of TRI from the sample processing

procedure. Those recovery values were subsequently factored into the calculation of TRI tissue concentrations.

It took less than 3 minutes for TRI concentrations in the inhalation exposure chamber to reach the target level of 3500 ppm after the start of introducing the chemical. The TRI concentrations were maintained at this level throughout the exposure period, with fluctuations within $\pm 5\%$.

The uptake of TRI into blood was rapid, with the TRI concentration increasing steadily over time and approaching near steady state within 40 minutes following the initiation of inhalation exposure in both milk- and water-dosed groups (Fig. 1A). The accumulation of TRI in the brain tissue increased steadily over the exposure period (Figure 1B). The highest TRI concentrations measured at 100 minutes in blood were 126.54 and 140.55 $\mu\text{g}/\text{ml}$ for treatment and control group, respectively, and in brain were 143.23 and 133.93 $\mu\text{g}/\text{g}$ tissue, respectively. In blood and in brain tissues, the two parameters of greatest interest relative to neurobehavioral toxicity with VOCs, TRI exhibited similar pharmacokinetic characteristics for both the treatment and the control groups, and there was no statistically significant difference in TRI concentrations of the two groups at any of the time points measured.

TRI concentrations in liver approached a near steady state equilibria for both milk- and water-dosed mice after approximately 60 minutes (Fig. 2A), as did the kidney TRI levels for the milk-dosed animals (Fig. 2C). The kidney concentrations of TRI in water-dosed animals appeared to be continually increasing over the exposure period, but there was no statistical difference

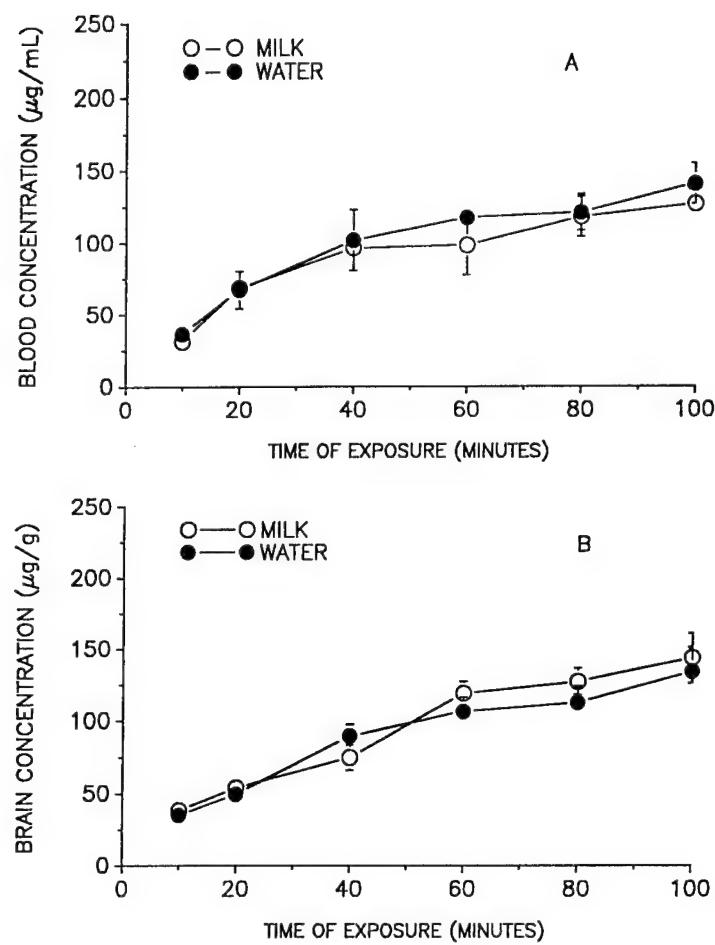


FIGURE 1

Concentrations of TRI in the A) blood and B) brain of mice following inhalation exposure to 3500 ppm for up to 100 minutes. Values are expressed as the mean \pm SE of 5 mice at each time point.

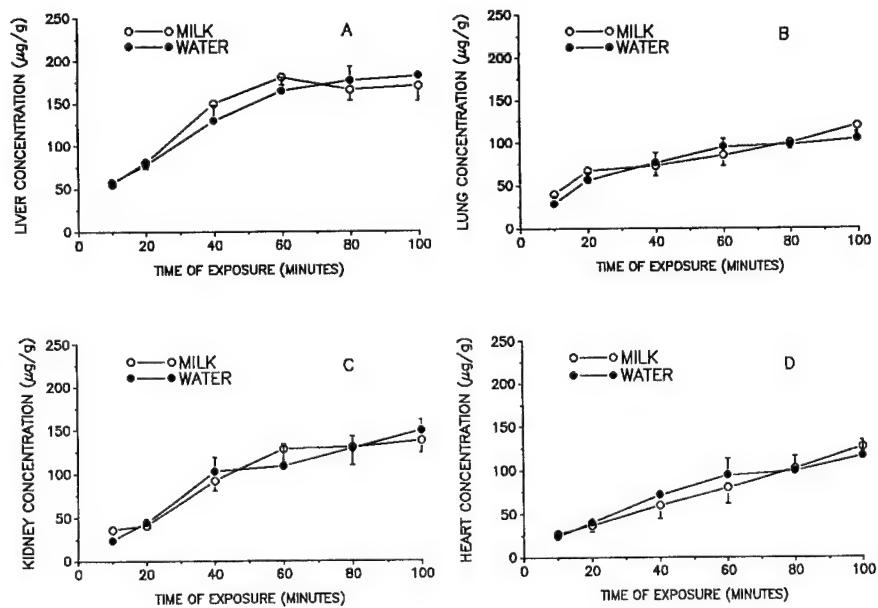


FIGURE 2

Concentrations of TRI in the A) liver, B) lung, C) kidney, and D) heart of mice following inhalation exposure to 3500 ppm for up to 100 minutes. Values are expressed as the mean \pm SE of 5 mice at each time point.

between the concentrations of the treatment and control groups for the kidney at any time point. TRI concentrations increased in a similar pattern over time in lung (Fig. 2B), heart (Fig. 2D), and spleen (Fig. 3C) for both milk- and water-dosed mice. There appeared to be a pattern of a higher TRI concentration in muscle (Fig. 3A) and GI (Fig. 3B) in the water-dosed animals relative to the mice receiving milk, especially at the terminal time point at 100 min. However, there was no statistically significant difference at any time point.

TRI showed a very different kinetic profile in fat tissue (Fig. 3D) than in the blood and other tissues. Fat TRI concentrations continued to rise considerably

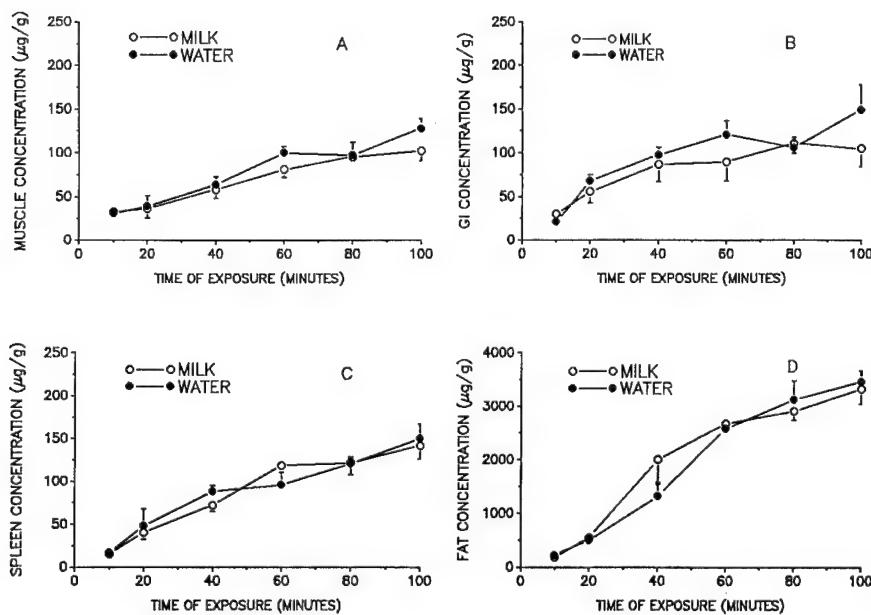


FIGURE 3

Concentrations of TRI in the a) muscle, b) GI, c) spleen, and d) fat of mice following inhalation exposure to 3500 ppm for up to 100 minutes. Values are expressed as the mean \pm SE of 5 mice at each time point.

over the course of the whole exposure period, with the highest concentrations measured at 100 minutes as 3326.8 and 3461.7 $\mu\text{g}/\text{g}$ for the treatment and control group, respectively. TRI concentrations in fat were about 20 to 30 times higher than the corresponding concentrations in the other tissues. It does appear that fat TRI concentrations in both of the milk- and water-dosed mice had approached a near-steady state after about 60 minutes, although the upward trend of the concentration curve did not disappear even at 100 minutes. This was also true in the majority of other tissues. There were no statistically significant differences

between TRI concentrations in fat tissues of milk- and water-dosed mice at any time point, though. The areas-under-the-curve (AUCs) for the blood and tissue concentration-time courses were calculated and plotted in Fig. 4. The blood and all nonfat tissues had similar AUC values for both milk- and water-dosed mice of approximately 10 mg·min/g, while the AUCs for fat tissue were close to 200 mg·min/g.

DISCUSSION

Studies of the pharmacokinetics of inhaled chemicals, especially VOCs, are playing an increasingly important role in toxicology^{24,25}. It has been suggested that the evaluation of endpoints in behavioral toxicology needs to be coordinated with pharmacokinetics as a sensible integration of behavior, physiology, and biochemical variables for VOCs²⁶. Operant performance tests have been one of the most useful neurobehavioral tests in detecting behavioral changes in laboratory animals following exposure to many VOCs, including TRI^{11,18}.

In conducting operant performance measurements, the use of milk presentation as a reinforcer has proven very effective in establishing stable operant behavior for the testing of neurobehavioral effects of VOCs¹⁶⁻¹⁹. In many of these studies, the repeated administration of milk over time has resulted in the animals receiving a considerable cumulative dose of milk over the course of the behavioral study. In operant measurements in rats conducted to evaluate the

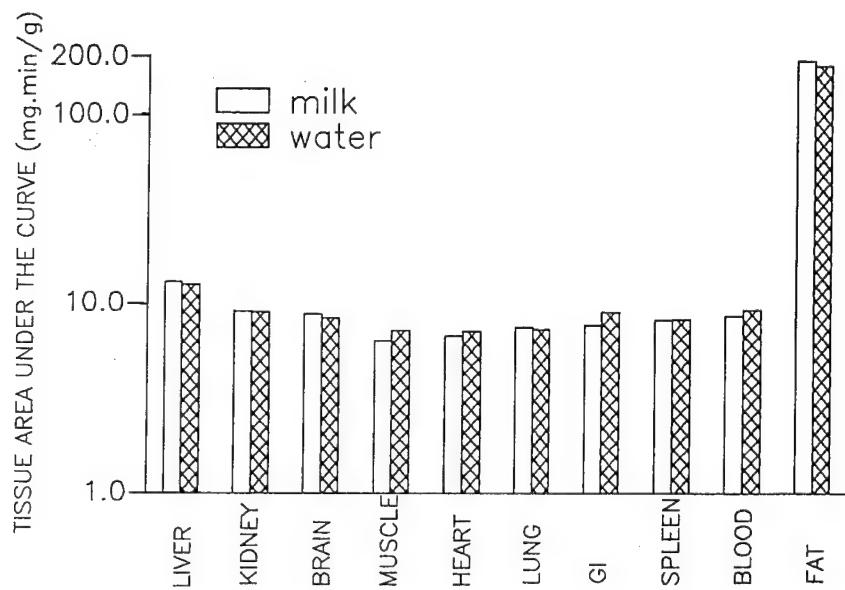


FIGURE 4

The areas under the blood and tissue concentration versus time curves for animals that inhaled 3500 ppm TRI for up to 100 minutes. The time interval is from 0 to 100 minutes.

neurobehavioral effects of TRI, Warren et al.¹⁵ typically administered more than 200 ml of milk to the test animals during the training periods. This would result in a body burden of about 660 g milk per kg of body weight (bw) of these animals as a result of the training protocol. In the current study, mice received approximately 21 ml over a three week period, to result in an administered dose of about 840 g milk/kg bw in mice.

Such a large addition of lipophilic components in the diet from this high degree of milk dosage would be expected to result in an increase in adipose tissue and lipid content in various organs. It has been found that the increase in body

fat in humans and animals that is concurrent with the aging process has been a very important factor in altering chemical distribution^{27,28}. A greater volume of distribution for chemical disposition would be expected to result for lipophilic compounds when there is an increase in adipose tissue. At the sites of action of these agents, this could mean that there could be a transiently lower concentration of lipophilic chemical like the great majority of VOCs.

However, an increase in body fat would be a pronounced increase in the body burden of these VOCs. Following repeated exposures of mice and rats to TRI, it is possible that the reported increased body burden of the compound was due to the concurrent increase in the volume of distribution of the fat compartment during that time period²⁹, as the high volatility and short half-life of the compound is known to typically result in its rapid elimination³⁰. It was shown that physiologically-based pharmacokinetic model simulations of TRI disposition were dramatically improved in comparison to experimentally observed data when the volume of the animal's body fat compartment was increased from 7 to 18% of body weight by computer optimization³¹.

In the current investigation, though, there were not any significant differences in the pharmacokinetics of TRI in the blood and nine tissues over a 100 minute time course of inhalation in the treatment and control groups of mice. In the blood and the brain, which would be expected to be the most important body compartments in relation to the neurobehavioral toxicity of VOCs, the time course of TRI following inhalation was remarkably similar between the mice that received water and those that were administered high doses of milk. The area

under the blood and tissue concentration versus time curves have been proposed as very feasible measures of target organ dose for experimental comparisons³², and there were only negligible differences between the two treatment groups for these parameters.

Although changes of the total fat volume of the animals was not monitored, their body weights were constant over time in both treatment and control animals. This was due to that fact that the food intake was compensated to maintain the body weight of the animals in order to correlate with the feeding protocol of the behavioral studies. It might have been expected that the most vivid effect of the milk dietary addition would have appeared in the fat compartment, and there both the milk- and water-dosed animals appeared to approach a near steady-state in TRI fat concentrations after approximately 60 minutes of TRI inhalation. As for individual nonfat organs, it has been proposed that a high lipid intake would increase lipid levels in the liver, which would enhance the target organ disposition there of lipophilic halocarbons³³. However, there were no significant differences between the milk- and water-dosed mice in liver TRI concentrations over the time course of the inhalation exposures employed in the current study.

There has been a paucity of tissue time course data published for VOCs to be used in comparison with toxicity data, such as the neurobehavioral depression associated with most of these compounds. TRI is a small, uncharged lipophilic molecule so it was readily absorbed across membranes of the pulmonary capillary bed into the systemic circulation, as evidenced by the rapid

appearance of the compound in all nine tissues monitored within 10 minutes of the initiation of inhalation exposure. As the chemical accumulated in the blood and tissues, the uptake became progressively slower. This was reflected in the approach to a near steady state equilibrium in most tissues after approximately 40-60 minutes of inhalation exposure to TRI.

It has been reported that the uptake and disposition of toluene²² and TRI¹⁵ in the blood and brain of rats was closely associated with the degree of neurobehavioral depression observed. It is reasonable to conclude, then, that the lack of the effect of the increased milk diet in mice on blood and tissue pharmacokinetics would preclude a pharmacokinetically related effect of milk diet on the neurobehavioral tests conducted in those animals. This would lend additional credibility to the continued use of milk as a reinforcer in neurobehavioral studies of lipophilic compounds such as VOCs.

ACKNOWLEDGEMENTS

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APPENDIX I

Warren, D.A., Reigle, T.G., Muralidhara, S., and Dallas, C.E. "Schedule-controlled operant behavior of rats following oral administration of perchloroethylene: time-course and relationship to blood and brain levels." *Toxicology and Environmental Health*, **47**: 101-118 1996.

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SCHEDULE-CONTROLLED OPERANT BEHAVIOR OF RATS FOLLOWING ORAL ADMINISTRATION OF PERCHLOROETHYLENE: TIME COURSE AND RELATIONSHIP TO BLOOD AND BRAIN SOLVENT LEVELS

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Previous studies have indicated that human exposure to perchloroethylene (PCE) produces subtle behavioral changes and other neurological effects at concentrations at or below the current occupational exposure limit. Since comparable effects in animals may be reflected by changes in schedule-controlled operant behavior, the ability of orally administered PCE to alter fixed-ratio (FR) responding for a food reward was investigated in male Sprague-Dawley rats. Furthermore, since behavioral effects of solvents are likely to be more closely related to blood or target tissue (i.e., brain) concentrations than administered dose, the relationship between the pharmacokinetic distribution of PCE and its effects on operant responding was also evaluated. Rats trained to lever-press for evaporated milk on an FR-40 reinforcement schedule were gavaged with 160 or 480 mg/kg PCE and immediately placed in an operant test cage for 90 min. Separate animals gavaged with equivalent doses of PCE were used to determine profiles of blood and brain concentrations versus time. Perchloroethylene produced changes in responding that varied not only with dose but also among animals receiving the same dose. Changes in the response rates of rats receiving 160 mg/kg PCE were either not readily apparent, restricted to the first 5 min of the operant session, or attributable to gavage stress and the dosing vehicle. However, 480 mg/kg produced either an immediate suppression of responding for 15–30 min before a rapid recovery to control rates or a complete elimination of lever-pressing for the majority of the operant session. Although the two doses of PCE produced markedly different effects on operant behavior during the first 30 min of exposure, differences in brain concentrations of PCE were minimal. Furthermore, the majority of animals receiving 480 mg/kg PCE fully recovered from response suppression while blood and brain levels of the solvent continued to rise. Thus,

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Results of this investigation were presented in part at the 31st Annual Meeting of the Society of Toxicology, Seattle, WA, February 1992. D. A. Warren was the recipient of a Department of Defense Science and Engineering Graduate Fellowship and an American Foundation for Pharmaceutical Education Fellowship.

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relationships between blood and brain PCE levels and performance impairment were not discernible over the monitored time course. Since the rapid onset of response suppression suggests that the precipitating event occurs within the first few minutes of exposure, it is possible that altered responding is related to the rate of increase in blood or brain concentrations rather than the absolute solvent concentrations themselves. The relationship between the pharmacokinetic distribution of solvents and their effects on the central nervous system is obviously complex and may involve acute neuronal adaptation as well as the dynamics of solvent distribution among the various body compartments.

Perchloroethylene (1,1,2,2-tetrachloroethylene, PCE) is a volatile organic solvent that is used in large quantities as a dry-cleaning agent, chemical intermediate, and metal degreaser (ATSDR, 1993). Approximately 650,000 workers in the United States are estimated to be at risk for occupational exposure to PCE (NIOSH/OSHA, 1990). Exposure surveys of the dry cleaning industry have determined that mean time-weighted average exposures typically range from 28.2 to 88.2 ppm (Materna, 1985) and from 4 to 149 ppm (Ludwig et al., 1983). Much higher exposures are associated with cleaning spills or replacing dry-cleaning filters (ATSDR, 1990). The 8-h time-weighted average workplace exposure limit for PCE is 100 ppm.

There is evidence that PCE is a rodent carcinogen (National Cancer Institute, 1977; National Toxicology Program, 1986), but such evidence in humans is equivocal. While PCE is a hepatic and renal toxicant at high doses, the majority of reports of human toxicity have focused upon neurological effects among those occupationally (Gregersen, 1988; Ferroni et al., 1992) or experimentally (Rowe et al., 1952; Stewart et al., 1970) exposed by inhalation. In these studies, acute exposures in the 100–200 ppm range were found to produce reversible mood changes and impaired coordination. Major electroencephalograph (EEG) changes suggestive of cerebral cortical depression have also been found among volunteers repeatedly exposed to 100 ppm (Hake & Stewart, 1977). Subchronic exposure to even lower PCE concentrations has reportedly caused memory loss and insomnia (Lauwerys et al., 1983), as well as perceptual, attention, and intellectual deficits (Seeber, 1989). Such studies suggest that PCE may have neurological effects at or below the current occupational exposure limit, some of which may be manifest as subtle behavioral changes.

While the ability of PCE to increase the ambulation (Savolainen et al., 1977) and motor activity (Kjellstrand et al., 1985; Fredriksson et al., 1993) of laboratory animals has been reported, PCE's effect on schedule-controlled operant behavior (SCOB) has not been investigated. Studies employing SCOB have, however, demonstrated behavioral effects for a number of other solvents, including toluene (Glowa et al., 1983; Wood et al., 1983), 1,1,2-trichloroethylene (Dews, 1978; Kulig, 1987) and 1,1,1-trichloroethane (Balster et al., 1982; Moser & Balster, 1986). Because the primary route of exposure to volatile solvents is inhalation, it was the original intent of this investigation to concurrently measure various PCE vapor concentrations and effects on SCOB. Attempts at doing so were unsuccessful, however, since

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rats trained to lever-press for evaporated milk immediately ceased responding upon exposure to even modest concentrations of PCE vapor. Case studies of an accidental poisoning victim (Koppel et al., 1985) and patients administered PCE as an anthelmintic agent (Wright et al., 1937; Sandground, 1941; Haerer & Udelman, 1964) have indicated that the acute neurological effects of PCE ingestion parallel those seen after inhalation. Therefore, SCOB was monitored after the oral administration of PCE. Because the behavioral effects of solvents may be more closely related to blood or target tissue (i.e., brain) concentrations than administered dose, the present study was designed to evaluate the relationship between the pharmacokinetic distribution of orally administered PCE and the effects of this agent on the SCOB of rats.

MATERIALS AND METHODS

Test Chemicals

Perchloroethylene of 99%+ purity was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Burdick and Jackson brand, high-purity solvent iso-octane was obtained from Baxter Healthcare Corp. (Muskegon, MI). Alkamuls EL-620 (formerly Emulphor EL-620), a polyethoxylated vegetable oil, was a gift of Rhone-Poulenc (Cranbury, NJ). Ten percent (160 mg/kg) or 20% (480 mg/kg) Alkamuls EL-620 was used to prepare stable emulsions of PCE in 0.9% saline. The actual concentration of PCE in each dosage formulation was confirmed by headspace gas chromatography.

Animals

Male Sprague-Dawley (SD) rats (Charles River Breeding Laboratories, Raleigh, NC) weighing 300–350 g were used in all experiments. Rats were housed 2 per cage in suspended wire-bottom cages (36 × 20 × 20 cm) in a temperature (22°C) and humidity (45%) controlled room with a 12-h light-dark cycle (light: 0700–1900 h). Rats were allowed to acclimate for a minimum of 7 d prior to use, during which time food (Purina lab chow 5001, Ralston Purina Co., St. Louis, MO) and tap water were available ad libitum. All experiments were conducted during the light phase of the light-dark cycle.

Operant Behavior

Twelve rats (6 per dose) were transferred to individual polypropylene cages (48 × 25 × 20 cm) with corncob bedding and stainless steel wire lids. Rats were food restricted during a period in which they were trained to lever-press for undiluted evaporated milk (0.05 ml for 7 s per reinforcement) on a fixed-ratio (FR) 40 reinforcement schedule (every 40th lever-press produced milk). Initially, rats were manually reinforced in 30-min sessions for coming near the lever or inadvertently touching it. Once rats learned to respond independently, the ratio of responses to reinforcers was gradually

increased to 40 and the session length extended to 90 min. Rats were allowed to respond in daily 90-min sessions spaced 24 h apart until their response rates stabilized, a process requiring 15–20 d. The criterion for stable behavior was 4 successive sessions in which the number of responses per second varied by less than 15%. Upon the completion of each operant session, rats were returned to their home cages and given 10 ± 0.25 g food, which was promptly eaten. Once rats exhibited stable behavior, they were gavaged with either a 10 or 20% aqueous Alkamuls vehicle (3 ml/kg), immediately placed in a modular test cage, and monitored for operant behavior for 90 min. Twenty-four hours later, rats were dosed with either 160 or 480 mg/kg PCE in the appropriate vehicle and their behavior was monitored again. Operant sessions took place in a test cage (Coulbourn Instruments, Inc., Lehigh Valley, PA) equipped with a house light, response lever, liquid delivery trough and dipper, and a stimulus light above the delivery trough that remained lit during availability of the milk reinforcer. The test cage was placed inside a 1.0-m³ Rochester-type dynamic flow inhalation chamber that served to isolate animals from extraneous stimuli. The test cage was interfaced via LabLinc (Coulbourn Instruments, Inc., Lehigh Valley, PA) with an IBM-compatible 386 computer running COSMOS software (Coulbourn Instruments, Inc., Lehigh Valley, PA) that applied the operant schedules and recorded the number of responses and reinforcers in each 5-min interval of the operant sessions.

Blood Sampling

Twelve rats (6 per dose) were food restricted (10 ± 0.25 g/d) for 72 h prior to being surgically implanted with an indwelling carotid artery cannula. Rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture of ketamine HCl (100 mg/ml), acepromazine maleate (10 mg/ml), and xylazine HCl (20 mg/ml) in the proportion 3 : 2 : 1 (v/v/v). The cannulae exited the skin at the nape of the neck and were protected from manipulation by surgical tape. After an overnight recovery period during which food was withheld (~18 h), either 160 or 480 mg/kg PCE was administered as an aqueous Alkamuls emulsion by bolus gavage in a total volume of 3 ml/kg. Following dosing, blood samples were withdrawn from the arterial cannulae of unrestrained and unanesthetized animals via a three-way stopcock and 1-ml syringe. Serial blood samples (2–75 μ l, depending upon the anticipated blood concentration) were taken at various times during the 4 d that followed dosing. Blood samples were quickly transferred to 8-ml headspace vials, capped immediately with Teflon-lined latex rubber septa in aluminum seals, and crimped tightly. Some blood samples required dilution with ice-cold saline in order to be analyzed within the linear range of the electron capture detector (ECD) of a gas chromatograph (GC). As necessary, blood withdrawal was followed by a heparin flush to maintain cannula patency. Food was available ad libitum during blood sampling.

Tissue Sampling

For the determination of PCE concentrations in tissues, rats were food restricted (10 ± 0.25 g/d) for 72 h, and subsequently fasted for 18 h prior to being dosed as described for blood sampling. Groups of 6 rats were sacrificed by cervical dislocation followed by decapitation at 1, 6, 15, 20, 30, 40, 50, 60, and 90 min after dosing. Approximately 0.5–1.0 g samples of brain, liver, perirenal fat, and skeletal muscle were excised within 2.5–3 min from each rat and immediately placed into chilled scintillation vials containing 2 ml of 0.9% saline and 8 ml isoctane. Tissues were homogenized as quickly as possible (5–15 s) with an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, OH) to minimize volatilization of PCE, after which the sample was vortex-mixed for 30 s. The homogenates were then centrifuged at $2500 \times g$ for 10 min at 4°C in the capped scintillation vials. An aliquot of the isoctane layer (5–20 μl) was either transferred directly to a 20-ml headspace vial or first diluted with ice-cold saline. The vials were capped immediately with Teflon-lined latex rubber septa in aluminum seals and crimped tightly.

PCE Analysis

A Sigma model 300 GC equipped with an HS6 headspace sampler and an ECD (Perkin-Elmer Co., Norwalk, CT) was used for the analysis of PCE in blood. Analyses were carried out using a stainless-steel column (182×0.317 cm) packed with 3% OV-17 (100–120 mesh) (Alltech Associates, Inc., Deerfield, IL). The GC operating conditions were: headspace sampler temperature, 80°C ; injection port temperature, 200°C ; column temperature, 110°C ; ECD temperature, 360°C ; flow rate for argon–methane (95 : 5) carrier gas, 60 ml/min. For PCE analysis of isoctane tissue extracts, a Perkin-Elmer model 8500 GC with an HS-101 headspace autosampler and ECD was employed under the same conditions as those previously listed. Perchloroethylene concentrations were calculated from daily prepared standard curves and were corrected for the percent recovery characteristic of blood and tissue samples. The percent recovery and blood/tissue extraction procedures have been described by Chen et al. (1993). The limit of detection for PCE was approximately 1 ng in 20 ml air.

Data Analysis

RSTRIP (version 3.1, 1988; MicroMath, Inc., Salt Lake City, UT) was used to fit PCE blood concentration versus time profiles to polyexponential equations for the calculation of area under the blood concentration versus time curve (AUC), elimination half-life ($t_{1/2}$), and maximum blood concentration (C_{\max}). Tests for differences in the concentrations of PCE in blood and tissues were made with two sample t-tests (T-ease Program, version 2.0, Institute for Scientific Information, 1987). The control behavioral response of each rat was calculated as the mean number of lever-presses in each 5-min interval of the 4 operant sessions used to meet the stability criterion.

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Time trends in control responses were examined by linear regression analysis (Wallenstein et al., 1980). One-way analysis of variance (ANOVA) was used to detect treatment-related differences in the responses of each rat. Repeated-measures analysis of variance (RMANOVA) was used to compare operant behavior between dose groups. Response ratios [vehicle/control (V/C), PCE/control (P/C), and PCE/vehicle (P/V)] for each of the eighteen 5-min intervals in the operant sessions were calculated so that each rat served as its own control. These ratios were subjected to a mixed-model RMANOVA with fixed factors of time and treatment and with a random factor of rat nested within treatment (Winer, 1971). In the event that time effects failed to satisfy multi-sample sphericity, test statistics for time effect and time \times treatment interaction were modified using the Huynh and Feldt adjustment factor (Huynh & Feldt, 1976). The RMANOVA was exclusive of rats 4B and 5B, both of whom failed to respond for the majority of the operant session following administration of 480 mg/kg PCE. Their exclusion did not change the conclusions drawn from variance analysis, but did modify *p* values. The minimum level of significance was set at *p* \leq .05 for all tests. On rare occasions, 5-min intervals of operant data were lost due to equipment malfunction.

RESULTS

Operant Behavior

It was determined in a dose range-finding study that the administration of \leq 80 mg/kg PCE by oral gavage had no effect on operant responding, whereas doses \geq 640 mg/kg resulted in occasional gait disturbances and a complete failure to lever-press. Two intermediate doses, 160 and 480 mg/kg, were thus selected for study. The operant responses of six rats from each of the two dose groups are shown in Figures 1 and 2. The control response rates of the low- and high-dose groups ranged from 1.04 to 3.04 (mean \pm SD, 1.88 \pm 0.75) and from 0.99 to 2.62 (1.63 \pm 0.58) responses per second, respectively. Neither group exhibited a significant trend in control responding; that is, control response rates did not have a tendency to significantly increase or decrease over the course of the operant sessions.

Response rates of the 2 dose groups averaged $91.6 \pm 5.2\%$ (mean \pm SD) and $111.0 \pm 18.2\%$ of control after administration of the 10 and 20% aqueous Alkamuls vehicles, respectively. One-way analysis of variance detected differences between the control and postvehicle responses of two of the twelve rats (3A and 6B). The failure of ANOVA to detect such a difference for rat 6A, when it is obvious that one exists, demonstrates its insensitivity to treatment-related effects when response rates are highly variable and effects are prevalent in only a portion of the overall operant session. Despite its insensitivity, variance analysis did detect PCE-induced changes from control and postvehicle responses that were much more prevalent in the high- than in the low-dose group.

Perchloroethylene-induced changes in responding of the low-dose group

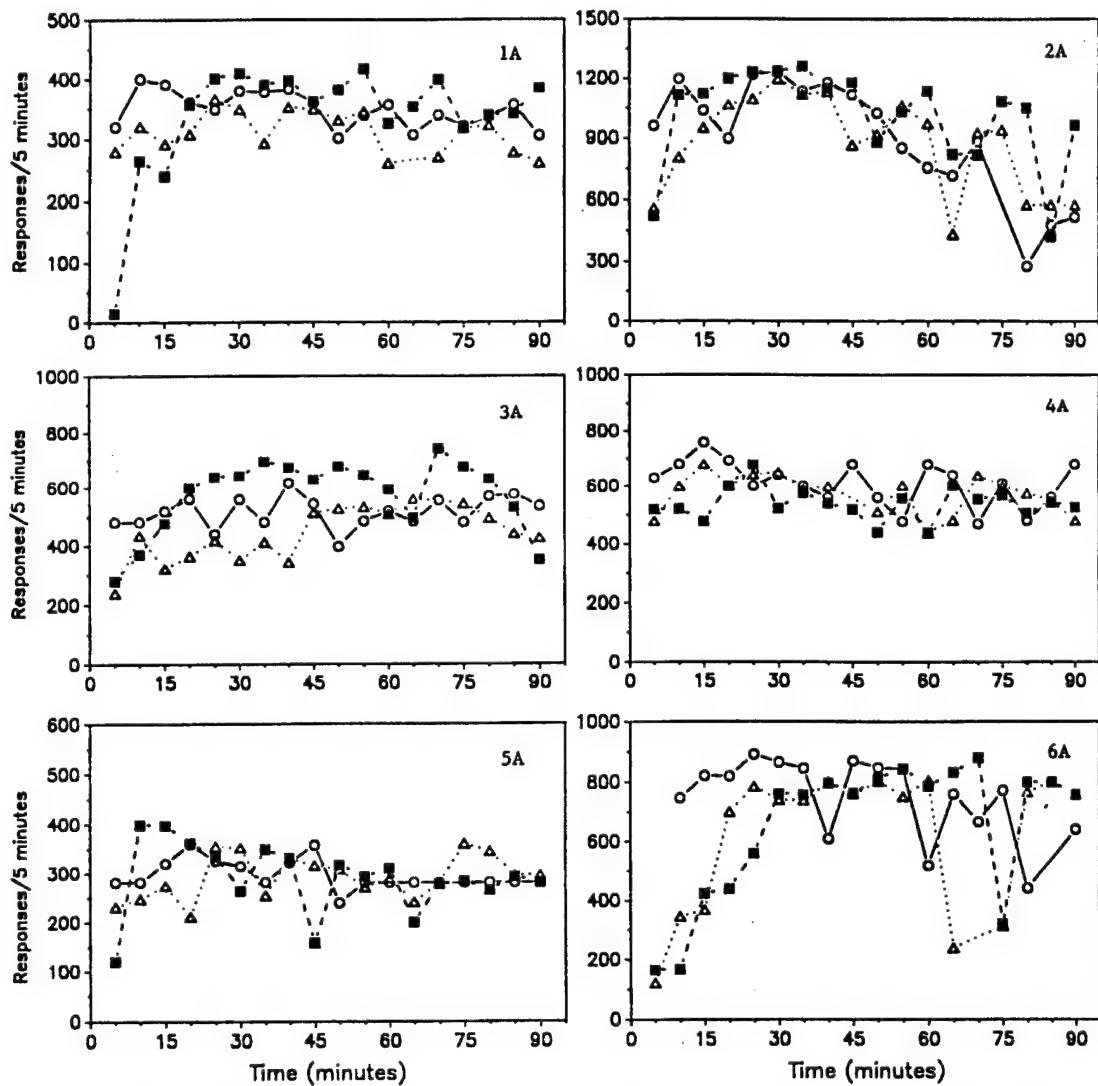


FIGURE 1. Operant responses of six rats following three treatments: no treatment or control (O); administration of a 10% Alkamuls vehicle (Δ); oral administration of 160 mg/kg PCE (\blacksquare).

were either not readily apparent (3A, 4A, and 5A), restricted to the first 5-min interval following dosing (1A), or attributable to gavage stress and the dosing vehicle (2A and 6A). In contrast, PCE's effects among the high-dose group included the immediate suppression of responding for 15–30 min before a rapid recovery to control or postvehicle response rates (1B, 2B, 3B, and 6B) or the complete elimination of lever-pressing for the majority of the operant session (4B and 5B).

The repeated-measures analysis of variance on V/C ratios failed to detect a significant time \times treatment interaction ($p = .335$), but did detect signifi-

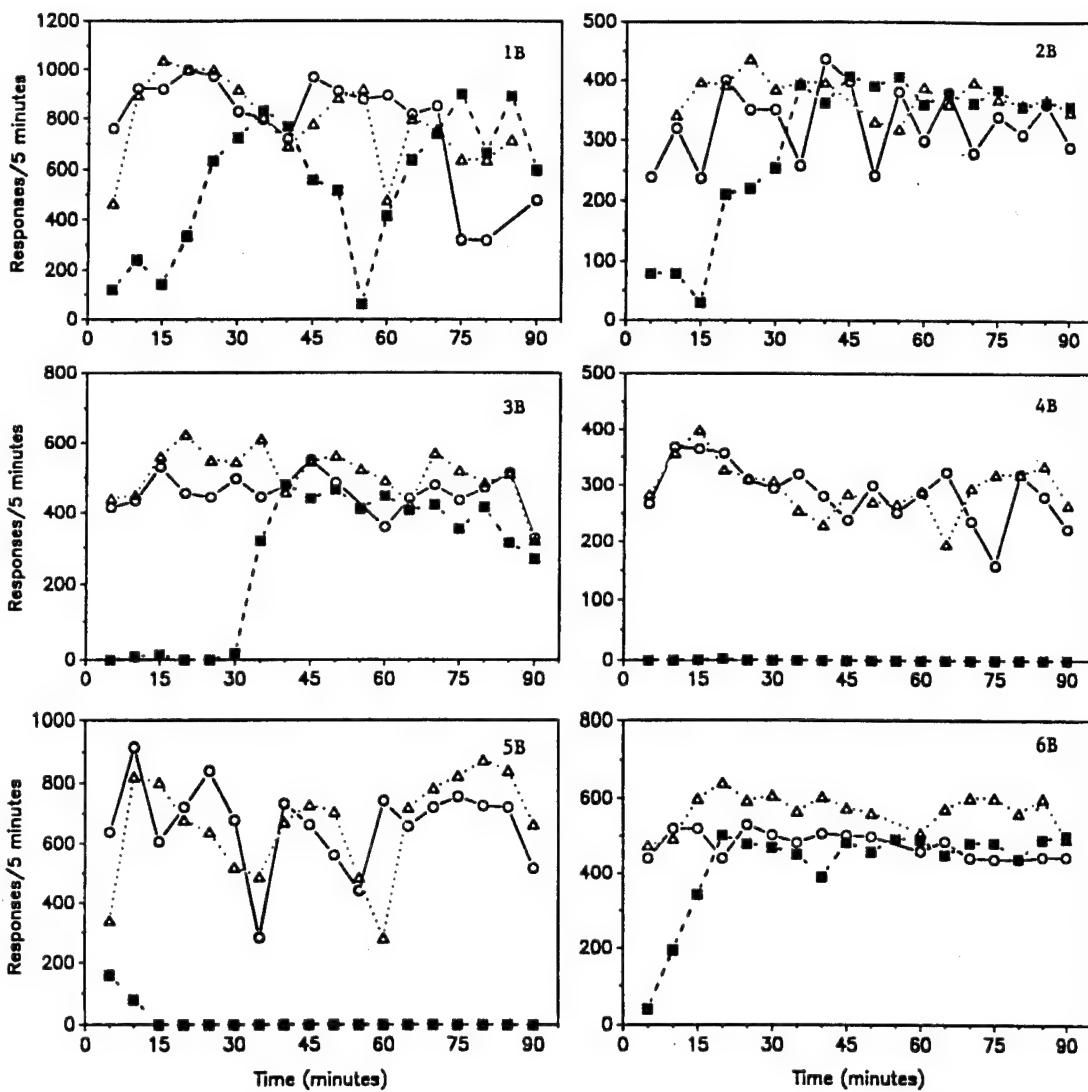


FIGURE 2. Operant responses of six rats following three treatments: no treatment or control (○); administration of a 20% Alkamuls vehicle (△); oral administration of 480 mg/kg PCE (■).

cant treatment ($p = .016$) and time ($p = .025$) effects. In general, the administration of the 10% aqueous Alkamuls vehicle reduced response rates, while the 20% vehicle increased response rates. This resulted in the high-dose group having higher mean V/C ratios during 16 of the 18 5-min operant session intervals. The vehicle effect was most apparent in the low-dose group immediately after dosing, at which time the response rates of all six animals were lower than control rates. Because of the significant vehicle effect, RMANOVA on both P/C and P/V ratios was performed. The analysis of P/C ratios did not detect a significant time \times treatment interaction ($p = .280$) or

treatment effect ($p = .187$), but did detect a significant time effect ($p = .0005$). When P/V ratios were submitted to variance analysis, there was no significant time \times treatment interaction ($p = .415$), but there was a highly significant treatment ($p = .001$) and time ($p = .023$) effect. The significant treatment effect stems from the suppression of operant responding by the 480-mg/kg dose of PCE, whereas the significant time effect is apparently due to the suppression of responding immediately after dosing, followed by increases in response ratios as the operant sessions progress. That RMANOVA detected a significant treatment effect on P/V and not P/C ratios is another indicator that vehicle administration affected responding. The differential effect of treatment on the two types of ratios may be due to response rates after low-dose administration differing more from control than from postvehicle response rates. Response rates following low-dose administration may have differed enough from control response rates that the effect of the high dose on P/C ratios was not significantly different.

New paragraph

Pharmacokinetics

The blood concentration versus time profiles following gavage with both PCE doses are presented in Figure 3. The long $t_{1/2}$ of PCE relative to that of most volatile halocarbons was demonstrated by its presence in blood for several days after dosing. The pharmacokinetic parameter estimates for each dose group are summarized in Table 1. The differences in AUC and C_{max} between the two doses were nearly proportional to the difference in PCE dose. A 3-fold increase in dose resulted in AUC and C_{max} values that were 2.7- and 3.6-fold higher, respectively. Blood $t_{1/2}$ did not differ with dose.

The blood and brain concentration versus time profiles for the 90 min immediately following dosing are presented in Figures 4 and 5, respectively.

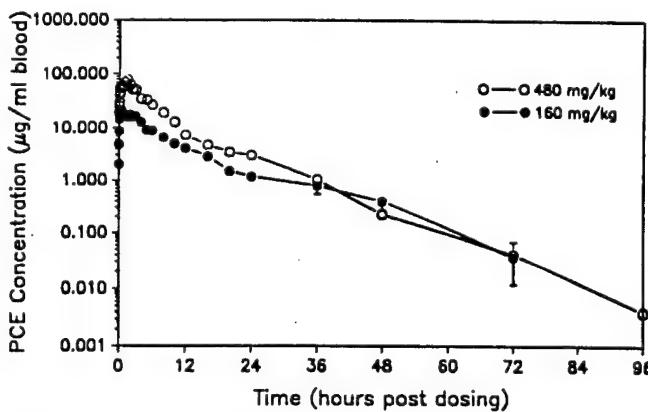


FIGURE 3. Blood PCE concentration versus time profiles following the oral administration of 160 and 480 mg/kg PCE. Each profile represents the mean \pm SE of six rats per dose. The concentration of PCE in blood at 96 h following administration of 160 mg/kg was below the limit of detection. SE bars are obscured by symbols in most cases.

TABLE 1. Pharmacokinetic parameters following oral administration of perchloroethylene to rats

Dose (mg/kg)	AUC ($\mu\text{g min/ml}$) ^a	$t_{1/2}(\text{min})^b$	$C_{\max} (\mu\text{g/ml})^c$
160	9508 \pm 730	504 \pm 46	21.5 \pm 2.8
480	25,256 \pm 1162	555 \pm 78	78.0 \pm 6.2

Note. Values are the mean \pm SE of six rats per dose.

^aArea under the curve that describes the concentration of PCE in blood as a function of time.

^bElimination half-life or the amount of time required for the blood concentration of PCE to be reduced by 50%.

^cMaximum concentration of PCE in carotid arterial blood.

This 90-min period corresponds to the time course over which the SCOB of rats was monitored. Perchloroethylene was rapidly absorbed from the gastrointestinal (GI) tract as evidenced by its presence in blood and brain as early as 1 min after dosing. Following a 10- to 15-min phase of very rapid PCE uptake by the blood and brain, uptake rates slowed as peak levels were approached. At 15 min following administration of 160 mg/kg PCE, blood and brain levels had reached 94 and 80% of their maxima, respectively, after which they were relatively stable out to 90 min. In contrast, at 15 min following administration of the high dose, blood and brain concentrations were only 74 and 42%, respectively, of the concentrations at 90 min.

In both the blood and brain, the ratio of PCE concentrations between doses was greatest at 1 min (9.5 in blood and 3.0 in brain). The blood PCE concentrations significantly differed between doses at all sampling times during the 90 min immediately following dosing ($p \leq .002$). Despite a threefold

less than 75

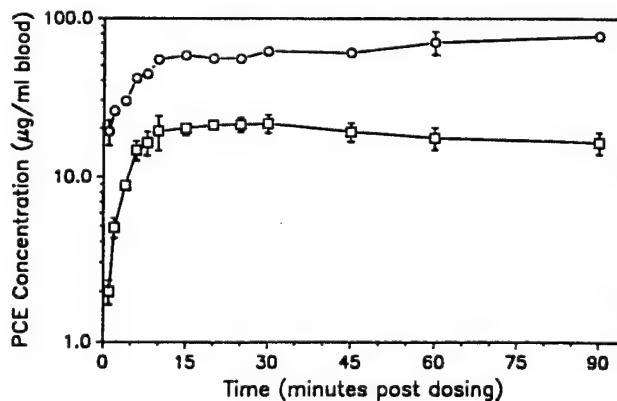


FIGURE 4. Uptake of PCE in blood during the 90 min immediately following oral administration of 160 (□) and 480 mg/kg PCE (○). Each profile represents the mean \pm SE of six rats per dose. SE bars are obscured by symbols in most cases.

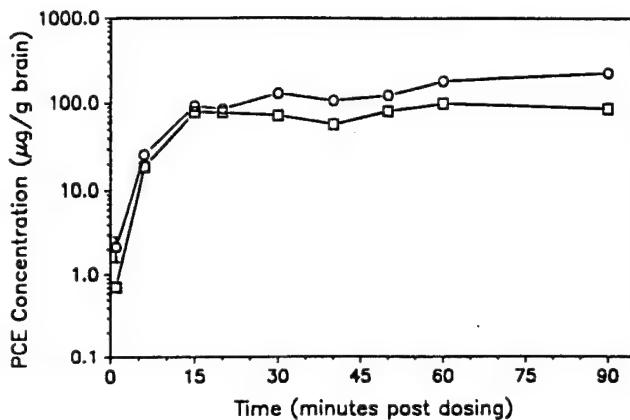


FIGURE 5. Uptake of PCE in brain during the 90 min immediately following oral administration of 160 (\square) and 480 mg/kg PCE (\circ). Each data point represents the mean \pm SE of six rats. SE bars are obscured by symbols in most cases.

difference in mean brain PCE concentrations at 1 min, the difference was not statistically significant ($p = .072$) due to variability within the high dose group. Brain concentrations at 6, 15, and 20 min also were not significantly different between doses ($p = .108$, $p = .214$, and $p = .449$, respectively), but were so at all subsequent sampling times ($p \leq .007$).

Tissue dose time courses for PCE were also determined in perirenal fat, liver, and skeletal muscle (Figure 6). Prior to 30 min postdosing, concentrations in these tissues rarely differed between doses. Apparently, the rate of blood perfusion and lipid content of these tissues had a significant impact on PCE deposition. Based upon relative tissue concentrations at 1 and 6 min, the well-perfused liver accumulated PCE at the highest rate, followed by the

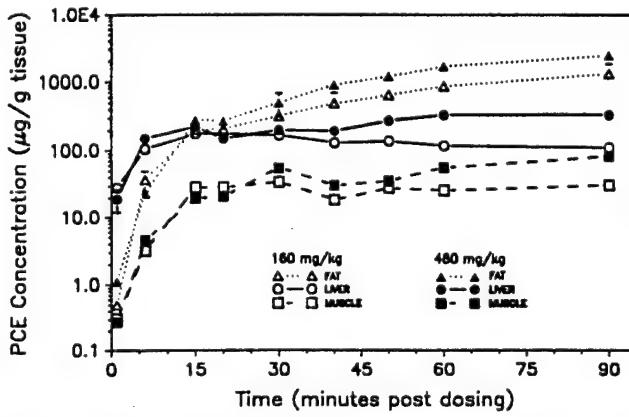


FIGURE 6. Uptake of PCE in perirenal fat, liver, and skeletal muscle during the 90 min immediately following oral administration of 160 and 480 mg/kg PCE. Each data point represents the mean \pm SE of six rats. SE bars are obscured by symbols in most cases.

fat and muscle. By 15 min, fat concentrations had exceeded those in the liver. At 90 min postdosing, at which time behavioral monitoring was discontinued, PCE concentrations were greatest in the fat, followed by the liver, brain, and muscle.

DISCUSSION

The investigation of possible relationships between solvent-induced central nervous system (CNS) effects and blood and brain solvent levels is important as a basis for predicting the time course of solvent toxicity, understanding differential susceptibility, elucidating sites and mechanisms of action, and extrapolating risk across species and exposure scenarios (Orr et al., 1976). With the exception of the large number of studies exploring the relationship between alcohol-induced CNS effects and blood ethanol levels (Sidell & Pless, 1971; Hurst & Bagley, 1972; Jones & Vega, 1972; Radlow & Hurst, 1985), few studies have attempted to relate solvent pharmacodynamics to pharmacokinetics. Bruckner and Peterson (1981) have demonstrated that blood and brain toluene concentrations are highly correlated with the degree of CNS depression in mice, as measured in tests of reflexes and unconditioned performance. Also, Kishi et al. (1993) have reported a relationship between blood 1,1,2-trichloroethylene levels and shock avoidance performance decrements in rats. In studies of controlled human exposures, impaired body balance, eye tracking deficits, and altered reaction times have been correlated with blood *m*-xylene or 1,1,1-trichloroethane concentrations (Riihimaki & Savolainen, 1980; MacKay et al., 1987).

Except in studies of ethanol in which oral dosing has been used almost exclusively, the CNS effects of solvents have typically been investigated after, and to a much lesser extent during, inhalation exposure. In the present study, however, PCE was administered orally at doses that were 4.1 and 12.5% of the LD₅₀ (Hayes et al., 1986). This was necessitated by the immediate arrest of lever-pressing by exposure to even modest concentrations (~500 ppm) of PCE vapor. Based on previous studies recognizing the possible disruptive effects of odor and irritant properties of solvent vapors on SCOB (Balster et al., 1982; Moser & Balster, 1986), we have attributed our observation of the cessation of lever-pressing to the odor or irritant properties of PCE. Burning of the eyes and irritation of the throat and nasal passages have been produced in humans by exposure to PCE in the 75–200 ppm range (ATSDR, 1990; NIOSH/OSHA, 1978), and the irritant potency of PCE in humans appears to be comparable to that of most industrial solvents. An exception appears to be the considerably less aversive 1,1,1-trichloroethane (Dick, 1988), and, while irritant potencies may differ for rats, it is noteworthy that 1,1,1-trichloroethane failed to disrupt operant behavior at concentrations well above those of PCE that abolished responding (Warren et al., 1993). Similarly, the possibility that disruptive effects on operant behavior may be produced by local irritant effects of PCE on the GI tract cannot be

dismissed, since patients receiving PCE as anthelmintic therapy have suffered abdominal cramps, nausea, vomiting, and lesions of the oral and gastric mucosa (Reichert, 1983). However, the observation that lever-pressing was merely suppressed and not fully extinguished by PCE in most rats suggests that GI irritation was not responsible for the behavioral effects observed in the present study.

The absorption and elimination pattern observed for PCE is characteristic of a lipid-soluble, poorly metabolized chemical. Similar solvents have been shown to be rapidly and completely absorbed from the GI tract, with peak blood levels occurring from 2 to 15 min postdosing (Reitz et al., 1982; D'Souza et al., 1985; Putcha et al., 1986). In the present study, PCE was quickly absorbed into the systemic circulation and taken up by body tissues. At 1 min postdosing, PCE was present not only in the well-perfused liver and brain, but also in the poorly perfused muscle and fat. This is in agreement with the observation of Dallas et al. (1994a) that peak levels for most tissues, including brain, occur at 1 min following intraarterial (ia) injection of PCE. Rapid uptake into tissues has also been reported following the autoradiographic analysis of mice after 10 min of ^{14}C -PCE inhalation (Ghantous et al., 1986). Additionally, relatively high arterial blood concentrations of PCE were present only 2 min after the initiation of inhalation exposures to rats (Dallas et al., 1994b). After oral administration, therefore, PCE follows a pattern of rapid uptake similar to that which follows administration by other routes.

Pegg et al. (1979) have reported the rapid and complete absorption of PCE after an oral bolus of 500 mg/kg in corn oil. These investigators observed a peak blood level of approximately 40 $\mu\text{g}/\text{ml}$, compared to a peak level in the current study of 78 $\mu\text{g}/\text{ml}$ following the delivery of 480 mg/kg as an aqueous emulsion. This discrepancy in peak blood levels can be attributed to the slower rate of absorption from the corn oil vehicle, allowing time for tissue uptake and elimination processes to reduce blood levels. The $t_{\frac{1}{2}}$ reported in the current study of approximately 9 h is comparable to previously reported values of 7.43 and 8.27 h for male SD rats (Pegg et al., 1979; Dallas et al., 1994a). Although an oral dose of 500 mg/kg PCE has reportedly resulted in the saturation of oxidative metabolism in the male SD rat (Pegg et al., 1979), there was no evidence of saturation in the current study as both the AUC and C_{\max} were roughly proportional to dose.

Little information exists on PCE that allows for comparisons between rat and human data and oral and inhalation exposures. The ingestion of 8–10 ml of PCE (545–727 mg/kg) by a 6-yr-old boy was followed by drowsiness, vertigo, agitation, hallucinations, somnolence, and coma, from which he made a full recovery (Koppel et al., 1985). One hour after ingestion, the PCE concentration in blood was 21.5 $\mu\text{g}/\text{ml}$, a level equal to the C_{\max} observed following the oral administration of 160 mg/kg to rats. Also, the oral administration of PCE as an anthelmintic commonly resulted in narcotic effects, inebriation, perceptual distortion, and exhilaration in patients receiving doses

ranging from 60 to 86 mg/kg (assuming a body weight of 70 kg) (Wright et al., 1937; Sandground, 1941; Haerer & Udelman, 1964). Regarding inhalation of PCE, Dallas et al. (1994a, 1994b) exposed rats to 500 ppm for 2 h and measured blood and brain concentrations during and after exposure. At 90 min, the blood concentration was about 20 $\mu\text{g}/\text{ml}$. The maximum brain concentration was 173.9 $\mu\text{g}/\text{g}$, which is about midway between the maximum brain concentrations that followed administration of the 2 doses in our study.

One might expect that PCE exposure would not affect operant response rates until a threshold concentration in the brain is reached, after which rate changes may be biphasic or monotonic in a decreasing direction. Only response-rate decreases would be expected in the current study, since an FR schedule typically generates near maximal response rates. It was therefore anticipated that response rates would steadily decline as blood and brain PCE levels increased. Instead, it appears that there are no discernible relationships between blood or brain PCE levels and performance impairment. For example, there was little difference in brain levels between doses prior to the 30-min sampling point, but drastic differences in operant behavior. Additionally, two-thirds of the rats in the high-dose group exhibited an immediate suppression of responding, followed by full recovery to control rates while blood and brain levels were still rising.

The rapid onset of response suppression suggests that the event that triggers behavioral effects occurs within the first 1 or 2 min after dosing. It is difficult to conclude, however, that the significant difference in behavior of the 2 dose groups was due to the relatively small difference in absolute brain concentrations at the 1-min sampling point [0.72 ± 0.08 (mean \pm SE) vs. $2.14 \pm 0.71 \mu\text{g}/\text{g}$]. Furthermore, it is evident that the behavior of the high-dose group was not solely determined by brain levels at 1 min, since concentrations up to 50-fold higher eventually occurred in the low-dose group. Also, the blood level in the low-dose group at 30 min (21.5 $\mu\text{g}/\text{ml}$) exceeded the blood level that occurred at 1 min in the high-dose group (19.1 $\mu\text{g}/\text{ml}$). It is possible that the response-rate changes were related to the rate at which concentrations increased, rather than to the absolute blood or brain concentrations themselves. Such has been suggested for diazepam- or *N*-desmethyl-diazepam-induced impairment of psychomotor skills (Linnoila & Mattila, 1973) and for *m*-xylene-induced body sway (Riihimaki & Savolainen, 1980). It is conceivable that the slower rise in blood and brain concentrations seen after the lower dose of PCE allowed the CNS time to adapt, thereby minimizing PCE-induced changes in operant responding.

At the end of the operant sessions, brain concentrations in the high-dose group were more than 100-fold higher than at 1 min, and 8-fold higher than at 6 min. Blood levels were generally two- to threefold higher when rats 1B, 2B, 3B, and 6B were responding at control rates than when their responding was severely suppressed. This phenomenon, which has been termed "acute adaptation," has been documented for ethanol and other CNS depressant drugs. Ellinwood et al. (1981a) have demonstrated that pentobarbital-induced

impairment of subcritical tracking, pendulum eye tracking, and standing steadiness in humans is much more marked during the initial phase of rapidly rising drug levels and is followed by rapid improvement of performance, despite rising drug concentrations in blood. In yet another study by Ellinwood et al. (1981b), peak impairment of wheel tracking and digit-symbol substitution performance in humans was seen 20 min after oral diazepam when blood levels had reached less than two-thirds of their eventual plateau. There was a reduction in this impairment over the next 40 min even though blood diazepam levels continued to increase. In perhaps the most noted study of this phenomenon (LeBlanc et al., 1975), the "acute adaptation" of rats to ethanol was demonstrated on a moving belt task. Impairment at a comparable brain level of ethanol was much greater 10 min after ip injection than at 30 min.

In a study similar to ours, Middaugh et al. (1992) examined lever responding in mice under an FR-20 food reinforcement schedule beginning 5 min after ip injection of ethanol. A dose of 1.5 g/kg reduced lever responding by 30% during the 0–4 min interval, but responding recovered to control rates by 5–8 min. Responding in mice given 2.0 g/kg was reduced 72 and 75% in the 5–8 and 9–12 min intervals, respectively, but had recovered to control levels by 13–16 min. Based on the current study and the work of Middaugh et al. (1992), it appears that a very rapid adaptation may occur to effects on reinforced behaviors. It is known that functional tolerance or acute adaptation may develop more readily when the effect of the agent has a behavioral cost to the experimental animal, such as when it reduces the capability of a food-restricted animal to obtain a food reward (Jaffe, 1990).

It is evident from this study, as well as others, that the relationship between the pharmacokinetic distribution of solvents and their effects on the CNS is complex. Such complexity may stem from nonlinearity between the administered dose of a solvent and the dose that reaches the brain, possible nonlinearity between the arterial blood and brain concentrations, and little-understood phenomena such as "acute adaptation." Given this complexity, it is becoming even more apparent that progress toward an understanding of mechanisms of solvent action in the CNS rests with performing studies that meet three criteria: (1) use of quantitative pharmacodynamic tests that allow for repeated measures over time in order to ascertain a detailed time course of effects, (2) use of highly sensitive and exact methods to assay blood and brain levels of solvents at repeated intervals, and, ideally, (3) the measurement of blood and brain levels of solvents in those animals undergoing neurobehavioral testing (Orr et al., 1976).

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APPENDIX J

Warren, D.A., Reigle, T.G., Christmus, W.H., Muralidhara, S., and Dallas, C.E. "Schedule-controlled operant behavior of rats during 1,1,1-trichloroethane inhalation: Relationship to blood and brain solvent concentrations." To be submitted to *Neurotoxicology and Teratology*.

SCHEDULE-CONTROLLED OPERANT BEHAVIOR OF RATS DURING 1,1,1-
TRICHLOROETHANE INHALATION: RELATIONSHIP TO BLOOD AND
BRAIN SOLVENT CONCENTRATIONS¹

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C.E. To be submitted to *Neurotoxicology and Teratology*.

ABSTRACT

The central nervous system is the principal target of 1,1,1-trichloroethane (TRI), and several studies of this volatile solvent have demonstrated effects on learned animal behaviors. There have been no attempts, however, to quantitatively relate such effects to blood or target organ (i.e., brain) solvent concentrations. Therefore, Sprague-Dawley rats trained to lever-press for evaporated milk on a variable interval-30 second reinforcement schedule were placed in an operant test cage and exposed to clean air for 20 minutes, followed by a single concentration of TRI vapor (500-5000 ppm) for 100 minutes. Additional rats were exposed to equivalent TRI concentrations for 10, 20, 40, 60, 80 or 100 minutes to determine blood and brain concentration versus time profiles. Inhalation of 1000 ppm slightly increased operant response rates, whereas 2000, 3500 and 5000 ppm decreased operant response rates in a concentration- and time-dependent manner. Accumulation of TRI in blood and brain was rapid and concentration-dependent, with the brain concentration roughly twice that of blood. Plots of blood and brain TRI concentrations against operant performance showed responding in excess of control rates at low concentrations, and decreasing response rates as concentrations increased. Linear regression analyses indicated that blood and brain concentrations were strongly correlated with, and equally predictive of, operant performance. Neurobehavioral toxicity in laboratory animals, as measured by changes in operant performance, can therefore be quantitatively related to internal measures of TRI exposure to enhance its predictive value for human risk assessment.

INTRODUCTION

1,1,1-Trichloroethane (methyl chloroform; TRI) is a volatile organic solvent used in large quantities as a dissolvent, metal degreaser, chemical intermediate and component

of consumer products. Originally produced as a safer alternative to other chlorinated solvents, the acute and chronic toxicities of TRI are relatively low. There is, however, a risk of toxic effects to those that encounter TRI in high concentrations in the workplace or recreationally abuse the solvent. It is thus noteworthy that an estimated 2.5 million U.S. workers are potentially exposed occupationally (ATSDR, 1994), and that solvent abuse has become a significant public health problem (Evans and Balster, 1991).

Severe exposures of humans to TRI have resulted in sensitization of the heart to epinephrine-induced arrhythmias and mild hepatorenal effects (ATSDR, 1994), but the central nervous system (CNS) is considered the principal target. Acute exposures to volunteers have produced impaired performance in tests of manual dexterity, eye-hand coordination, perceptual speed and reaction time (Gamberale and Hultengren, 1973; Mackay *et al.*, 1987). Some of these deficits have occurred at concentrations at or below the 8-hour, time-weighted average workplace exposure limit of 350 ppm. Less subtle effects such as lightheadedness and imbalance are usually observed at concentrations \geq 900 ppm (Torkelson *et al.*, 1958; Stewart *et al.*, 1961).

Studies of operant behavior are thought to reflect effects of TRI in laboratory animals comparable to psychomotor changes in humans. Accordingly, TRI has produced rate changes in the food-reinforced lever-pressing of mice (Balster *et al.*, 1982; Moser and Balster, 1986), altered performance on a match-to-sample discrimination task in baboons (Geller *et al.*, 1982) and impaired the ability of rats to avoid shock by lever-pressing (Mullin and Krivanek, 1982). These studies demonstrate that TRI's effects are concentration- and time-dependent, thereby inferring dependence on blood and target tissue (i.e., brain) doses. In no case, however, was blood or brain concentration measured for correlation with observed effects.

Except for the large number of studies exploring the relationship between alcohol-induced CNS effects and blood ethanol levels (Sidell and Pless, 1971; Hurst and Bagley, 1972; Jones and Vega, 1972; Radlow and Hurst, 1985), few studies have attempted to

correlate solvent pharmacodynamics with pharmacokinetics. This is unfortunate since such studies provide a basis for predicting the time-course of solvent toxicity, understanding differential susceptibility and extrapolating risk across species and exposure scenarios (Orr *et al.*, 1976). We report results of an investigation designed to examine the relationship between blood and brain TRI concentrations and changes in the schedule-controlled operant behavior (SCOB) of rats. 1,1,1-Trichloroethane was selected for study because it undergoes minimal metabolism and is psychoactive in humans and rodents. Furthermore, blood TRI concentration data are available from both controlled human and animal exposures. Schedule-controlled operant behavior was employed since it allows for repeated, uninterrupted measures over time that are sufficiently quantitative for correlation with measures of internal dose.

MATERIALS AND METHODS

Chemicals: 1,1,1-Trichloroethane of 97% + purity was obtained form Aldrich Chemical Co., Inc. (Milwaukee, WI). Burdick and Jackson Brand, High Purity Solvent isoocane was obtained from Baxter Healthcare Corp. (Muskegon, MI).

Animals: Male Sprague-Dawley (SD) rats (275-350 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC). Rats were housed two per cage in suspended wire-bottom cages (36 x 20 x 20 cm) in a temperature- (22°C) and humidity- (45%) controlled room with a 12-hour light-dark cycle (light: 0700-1900 hr). Rats were acclimated for at least 7 days prior to use, during which time food (Purina Lab Chow #5001, Ralston Purina Co., St. Louis, MO) and tap water were available *ad libitum*. All experiments were conducted during the light phase of the light-dark cycle.

TRI Vapor Generation: 1,1,1-Trichloroethane exposures were conducted in a 1.0 M³ Rochester-type dynamic flow inhalation chamber operating at a flow rate of 14 ft³/minute, under a negative pressure of 20" H₂O. Nitrogen was passed through a glass

dispersion flask of liquid TRI from which solvent vapor entered the chamber's influent air stream. A heating mantle was placed around the dispersion flask to generate vapor concentrations \geq 2000 ppm. The flask was enclosed in a plexiglass safety box under constant negative pressure. Exhaust air from the inhalation chamber and safety box was vented through HEPA and activated charcoal filters prior to its release into the environment. Vapor concentrations were continuously monitored with a Miran 1B2 portable infrared spectrophotometer ($\lambda = 9.4 \mu\text{m}$) (The Foxboro Co., East Bridgewater, MA) interfaced with a microcomputer-based Foxboro DL 332F Datalogger (Metrosomics Inc., Rochester, NY). The Miran was calibrated with a closed loop system (The Foxboro Co., East Bridgewater, MA) and the calibration accuracy verified just prior to each exposure with liquid TRI injections that volatilized to produce concentrations spanning the calibration range. Target vapor concentrations were reached within 2-5 minutes and thereafter exhibited $\pm 5\%$ random fluctuation. Occasional adjustments in nitrogen flow and heating mantle temperature were necessary to maintain target concentrations.

Behavioral Apparatus: Operant sessions took place in a slotted test cage (Coulbourn Instruments, Inc., Lehigh Valley, PA) positioned inside a 1.0 M³ Rochester-type dynamic flow inhalation chamber that served not only to expose animals, but also to isolate them from extraneous stimuli. This test cage was equipped with a house light, response lever, liquid delivery trough and dipper, and a stimulus light above the delivery trough that remained lit during the availability of the milk reinforcer. The test cage was interfaced via LabLinc (Coulbourn Instruments, Inc., Lehigh Valley, PA) with an IBM-compatible 386 computer running COSMOS software (Coulbourn Instruments, Inc., Lehigh Valley, PA) that applied the operant performance schedule and recorded the number of responses and reinforcers in each 5-minute interval of the operant session.

Operant Behavior: Rats were transferred to individual polypropylene cages (48 x 25 x 20 cm) with corn cobb bedding and stainless steel wire lids. Rats were food-restricted

(10 ± 0.25 g/day) during a period in which they were trained to lever-press for undiluted evaporated milk (0.08 ml for 7 seconds/reinforcement) on a variable interval-30 second (VI-30) reinforcement schedule. Initially, rats were manually reinforced in 30-minute sessions for coming near the lever or inadvertently touching it. Once rats learned to respond independently, the mean interval between reinforcer availability was gradually increased to 30 seconds and the session length extended to 120 minutes. Rats responded in daily 120-minute sessions spaced 24 hours apart until their response rates stabilized, a process requiring 15-20 days. The criterion for stable behavior was four successive sessions in which the number of responses/second varied by less than 15% from the 4-day mean rate. Once rats met the stability criterion, their behavior was monitored during exposure to clean air for 20 minutes, followed by a single concentration of TRI vapor (500, 1000, 2000, 3500 or 5000 ppm) for 100 minutes. Five chemically-naive rats were exposed to each TRI concentration.

Blood and Brain Sampling: Rats were transferred to individual wire-bottom cages (36 x 20 x 20 cm), food restricted (10 ± 0.25 g/day), and orally gavaged with 10 ml/day (two 5 ml boli given 1 hour apart) of undiluted evaporated milk for 15-20 days. This provided a diet comparable to that of rats in the behavioral study. At the end of the 15-20 day period, groups of five to twenty rats were placed in partitioned wire-mesh cages positioned in the same inhalation chamber employed in the behavioral study. Thirty rats were exposed to 1000 ppm TRI, five for each of the following durations: 10, 20, 40, 60, 80 and 100 minutes. Thirty rats were similarly exposed to 2000, 3500 and 5000 ppm TRI. At appropriate times during exposure, rats were extracted from the chamber through portals in the glass door with only a slight, transient decline in chamber concentration. The extracted rats were sacrificed by cervical dislocation after which blood samples (0.1-1.0 ml, depending upon the anticipated blood concentration) were quickly obtained by closed chest cardiac puncture with a 21 gauge needle and a 10 ml syringe. The brain (≈ 1.0 g samples) was removed within 2.5-3.0 minutes from each

rat and like the blood sample, immediately placed into a chilled scintillation vial containing 1.0 ml of 0.9% saline and 4.0 ml of isoctane.

TRI Analysis: Blood and brain samples were homogenized as quickly as possible (5-10 seconds) with an Ultra-Turrax® homogenizer (Tekmar Co., Cincinnati, OH) to minimize volatilization of TRI. Samples were then vortex-mixed for 30 seconds. The homogenates were centrifuged at 2500 x g for 10 minutes at 4°C in the capped scintillation vials. An aliquot of the isoctane layer (3-20 µl) was either transferred directly to a 20 ml headspace vial or first diluted with isoctane. The vials were capped immediately with Teflon®-lined latex rubber septa in aluminum seals and crimped tightly. A Perkin-Elmer Model 8500 gas chromatograph with a HS-101 headspace autosampler and electron capture detector was used for the analysis of TRI in blood and brain. Analyses were carried out using a stainless-steel column (182 x 0.317 cm) packed with 3% OV-17 (100-120 mesh) (Alltech Associates, Inc., Deerfield, IL). The GC operating conditions were: headspace sampler temperature, 70°C; injection port temperature, 150°C; column temperature, 80°C; ECD temperature, 360°C; flow rate for argon:methane (95:5) carrier gas, 60 ml/minute. 1,1,1-Trichloroethane concentrations were calculated from daily prepared standard curves made by diluting various amounts of TRI in isoctane and corrected for the percent recovery characteristic of blood and brain samples. The percent recovery and blood/brain extraction procedures have been described by Chen *et al.* (1993). The limit of detection for TRI was approximately 1 ng in 20 ml air.

Data Analysis: Areas-under-blood- and brain-concentration versus time curves (AUC) were calculated by the trapezoidal rule (Rowland and Tozer, 1980). Maximum blood and brain concentrations (C_{max}) were obtained by visual inspection of pharmacokinetic data. Each animals control behavior was calculated as the average number of lever-presses in each 5-minute interval of the four operant sessions used to meet the stability criterion. Time trends in control behavior were examined by linear regression analysis

(Wallenstein *et al.*, 1980). The effect of TRI in individual rats was determined by calculating an operant response ratio (lever-presses during a test session/lever-presses during control behavior; TRI/Control) for each 5-minute interval of a test session. Operant response data are presented as the mean \pm standard error (SE) of these individual determinations and as a function of exposure concentration. Operant response ratios during TRI exposure were subjected to a mixed-model repeated measures analysis of variance (RMANOVA) with fixed factors of time and concentration, and a random factor of rat nested within concentration (Winer, 1971). In the event that time effects failed to satisfy multisample sphericity, test statistics for time effect and time \times concentration interaction were modified using the Huynh and Feldt adjustment factor (Huynh and Feldt, 1976). One-way analysis of variance (ANOVA) and a *post hoc* "least significant difference" (LSD) test were applied to the mean operant response ratios. Paired t-tests were also used to compare the number of responses under control and test conditions. Pooling data from four exposure groups (1000, 2000, 3500 and 5000 ppm), blood and brain concentrations were plotted against each other, as well as against mean operant response ratios measured during the 5-minute intervals immediately preceding and following blood and brain collection. The resulting scatter plots, as well as the curve relating exposure concentration to operant responding, were subjected to least squares linear regression analysis and the degree of correlation measured by comparing correlation coefficients to values in a t-distribution table (Gad and Weil, 1986). EC₅₀ values (blood, brain and exposure concentrations expected to decrease responding by 50%) were determined by solving equations for the regression lines, and the confidence interval (CI) was defined using SAS (SAS Institute, Cary, NC). The minimum level of significance was set at $p \leq 0.05$ for all tests. Measures of variation are standard deviations (SD) unless otherwise specified.

RESULTS

Operant Behavior

Control response rates of the 500, 1000, 2000, 3500 and 5000 ppm exposure groups were 0.31 ± 0.15 , 0.31 ± 0.17 , 0.46 ± 0.10 , 0.36 ± 0.08 and 0.44 ± 0.11 responses/second, respectively. Control response rates did not have a tendency to significantly increase or decrease over the course of operant sessions. Response rates during the 20 minutes preceding TRI exposure were $\pm 25\%$ of control rates for 20/25 rats, and within ± 2 SD of control rates for the remaining five. Nonetheless, for the 500 ($t = 2.346$, $df = 19$, $p = 0.030$) and 5000 ppm ($t = 3.054$, $df = 19$, $p = 0.007$) exposure groups, paired t-tests detected that response rates during the 20 minutes preceding TRI exposure were higher than control response rates for the same period.

Mean operant response ratios (\pm SE) of the five exposure groups over time are shown in Figures 1a-1e. Operant response ratios during TRI exposure averaged 1.20 ± 0.15 , 1.20 ± 0.17 , 0.83 ± 0.22 , 0.53 ± 0.14 and 0.15 ± 0.16 for the 500, 1000, 2000, 3500 and 5000 ppm exposure groups, respectively, on which basis the dose-response curve in Figure 2 was composed ($EC_{50} = 3577$ ppm, 95% CI = 2864-4253 ppm). Repeated measures analysis of variance detected a significant time \times concentration interaction ($F(76, 380) = 1.50$, $p = 0.037$) and concentration effect ($F(4, 20) = 28.63$, $p < .0001$), but no time effect ($F(19, 380) = 1.44$, $p = 0.162$). One-way ANOVA followed by a *post hoc* LSD test also detected the effect of concentration ($F(4, 95) = 140.20$, $p < 0.0001$), as well as that operant response ratios of all exposure groups during TRI inhalation differed from unity ($p < 0.01$) and each other ($p < 0.01$), with the exception of the 500 and 1000 ppm exposure groups which did not differ. In addition, paired t-tests detected response rate increases during exposure to 500 ($t = 5.103$, $df = 99$, $p < 0.0001$) and 1000 ppm ($t = 4.376$, $df = 99$, $p < 0.0001$), and response rate decreases during exposure to 2000 ($t = 4.193$, $df = 99$, $p < 0.0001$),

3500 ($t = 12.245$, $df = 99$, $p < 0.0001$) and 5000 ppm ($t = 25.059$, $df = 99$, $p < 0.0001$). Despite statistical indices that 500 ppm increased operant responding, derivation of this conclusion was hindered by the elevated response rate exhibited by the 500 ppm group during the 20-minute pre-exposure period (Figure 1a). Mean operant response ratios of the 500 and 1000 ppm exposure groups were nearly identical, but subjects in the latter group demonstrated more variable response rates during exposure, which may itself be indicative of increased toxicity. Response rate decreases were concentration- and time-dependent. Decreased response rates were not exhibited until after 60 minutes of exposure to 2000 ppm. However, inhalation of 3500 ppm resulted in an immediate, but gradual decline in the average response rate to approximately 50% of control, while inhalation of 5000 ppm produced a precipitous drop in the response rate to approximately 15% of control. Only during exposure to this highest concentration did rats cease responding for periods in excess of 5 minutes, during which the animals typically assumed an immobile posture in close proximity to the lever. Rats did not exhibit ataxia or signs of irritation of the eyes, nose or mouth, and animals exposed to 5000 ppm returned to pre-exposure response rates within 15-25 minutes of exposure cessation.

Pharmacokinetics

Changes in TRI concentrations in the blood and brain as a function of degree and duration of exposure are shown in Figures 3a and 3b, respectively. These concentration versus time profiles correspond to the time-course over which the SCOB of rats was monitored. 1,1,1-Trichloroethane was rapidly absorbed from the lung as evidenced by its substantial presence in blood and brain as early as 10 minutes after dosing. Following a 20 to 40-minute phase of very rapid TRI uptake by the blood and brain, the rate of increase slowed as near steady-state equilibria between alveolar, blood and brain TRI concentrations were approached. Steady-state equilibria were apparently achieved during the 1000 and 2000 ppm exposures, while TRI concentrations were still increasing after

100 minutes of exposure to 3500 and 5000 ppm. Maximum blood and brain concentrations were proportional to exposure concentrations, while AUCs became slightly less than proportional at 3500 and 5000 ppm (Figures 4 and 5).

As expected for a well-perfused and lipid-rich organ such as the brain, its pattern of TRI accumulation was very similar to that of the blood. The scatter plot relating mean blood and brain solvent concentrations (Figure 6) clearly illustrates that brain levels of TRI increase in proportion to blood levels ($\approx 2:1$), making blood and brain solvent concentrations highly correlated ($r = 0.983$, $df = 21$, $t = 24.53$, $p < 0.001$). These findings strongly suggest that blood and brain TRI concentrations are equally suited to relate to operant performance measures.

Pharmacodynamic/Pharmacokinetic Correlation

The scatter plots relating mean blood and brain solvent concentrations to operant performance are presented in Figure 7. Responding was in excess of control rates at low blood and brain concentrations, and decreased as blood and brain concentrations increased. Linear regression analyses indicated that blood ($r = -0.75$, $t = 0.4375$, $df = 19$, $p < 0.001$) and brain ($r = -0.75$, $t = 0.4345$, $df = 19$, $p < 0.001$) concentrations were strongly correlated with, and equally predictive of, operant performance. Blood and brain EC₅₀ values were 41.6 $\mu\text{g}/\text{ml}$ and 86.6 $\mu\text{g}/\text{g}$, respectively.

DISCUSSION

Comparisons between the present and previous studies of TRI's effects on operant behavior are limited by the use of different species, exposure parameters and reinforcement schedules. However, the inhalation EC₅₀ of 3577 ppm obtained in the present study is comparable to those reported in two previous investigations of fixed ratio responding in mice during TRI exposure (Balster *et al.*, 1982; Moser *et al.*, 1985). Although the effect of TRI on the operant behavior of rats has not been previously

reported, the minimum effective concentration obtained in the present study is similar to those reported in rats to elicit changes in flash-evoked potentials and electroencephalograms (ATSDR, 1994), and well below levels required for unconditioned reflex failure, increased motor activity and ataxia (Clark and Tinston, 1982; Mullin and Krivanek, 1982; ATSDR, 1994).

In the present study, both changes in operant behavior and blood and brain concentrations of TRI were functions of the degree and duration of exposure. As expected for a lipid soluble chemical with a relatively low blood:air partition coefficient, the absorption pattern of TRI was characterized by very rapid uptake, with a rapid approach to steady state. Data from experiments in animals and humans provide supporting evidence that TRI is rapidly absorbed by the respiratory system. Dallas *et al.* (1989) have reported that arterial blood levels of TRI were quite high in rats within 2 minutes of exposure to 50 or 500 ppm. Furthermore, TRI was detected in the arterial blood of men within 10 seconds of exposure to 250 or 350 ppm (Astrand *et al.*, 1973). Once absorbed, TRI distributes among the various organs in proportion to organ blood flow and organ lipid content (Baker and Fine, 1986). As a result, TRI rapidly and extensively accumulates in the lipid-rich rat brain which is estimated to receive 2.21% of cardiac output while composing only 0.6% of total body weight (Dallas *et al.*, 1994).

Unfortunately, it cannot be assumed that animals and humans will react similarly upon inhaling the same TRI concentration since physiological differences exist among species that ultimately influence the amount and time-course of TRI deposition in the brain. For example, mice and rats have higher TRI blood:air partition coefficients than humans, and thus experience greater systemic uptake of TRI (Reitz *et al.*, 1988). Mice and rats also have higher respiratory and circulatory rates, two additional factors that contribute to a greater body burden. The impact of these physiological differences on TRI kinetics can be dramatic (Schumann *et al.*, 1982). For example, when normalized for differences in exposure concentration, the blood TRI levels of mice and rats reported

by Schumann *et al.* (1982) were 17.3 and 3.5 times those reported in humans by Nolan *et al.* (1984). The extrapolation of behavioral dose-response data generated in rodents, in the absence of comparative pharmacokinetic analysis, may therefore overstate human risk. As a result, it has been suggested that a scientifically-defensible approach to making interspecies extrapolations would assume that a particular target tissue dose in one species is equally as toxic in another (Andersen, 1987). Such an approach would benefit greatly from dose-response relationships where brain dose or a suitable dose surrogate is correlated with behavioral changes.

Because blood and brain concentrations in the present study were strongly correlated and equally predictive of operant performance, the blood TRI concentration would appear to be a suitable surrogate for brain concentration, provided sufficient time has elapsed for blood and brain concentrations to equilibrate. This is supported by the findings of Ameno *et al.* (1992) who showed strong correlations and linear relationships between blood and regional brain concentrations of toluene in rats. In addition, Bruckner and Peterson (1981) have concluded that blood concentration is a reasonable index of the depth of toluene-induced narcosis in mice as measured in tests of reflexes and unconditioned performance. In the present study, blood and brain concentrations of TRI were selected as dose metrics since 1) the very limited biotransformation of TRI makes metabolites of little concern to the CNS; 2) TRI's behavioral effects are thought to reflect the consequences of neuronal membrane fluidization, the degree of which is proportional to the amount of TRI dissolved therein; and 3) brain concentrations of TRI appear to be directly dependent on blood concentrations.

There appear to be important limitations, however, to the use of blood and brain concentrations as dose metrics in the present study. For example, after 60 minutes of exposure to 2000 ppm TRI, blood and brain concentrations equaled or exceeded those after 10 minutes of exposure to 5000 ppm. However, the 2000 ppm exposure group was unaffected 60 minutes into exposure, while the 5000 ppm exposure group exhibited a

drastic decline in response rate shortly after exposure initiation. This indicates that response rate suppression is not solely related to blood and brain concentrations, but may also be dependent upon the rate of TRI uptake. The rate of uptake has previously been implicated as a factor in m-xylene-induced body sway (Riihimaki and Savolainen, 1980) and diazepam-induced impairment of psychomotor skills (Linnoila and Mattila, 1973).

Insight into the species generality of the relationship between brain concentration and operant performance can be gained by examining the results of a parallel study to the present one, which employed CD-1 mice and a VI-60 reinforcement schedule (You *et al.*, 1994). Blood concentrations of TRI in mice were 2- to 3-fold higher than in rats during exposure to 3500 and 5000 ppm, yet brain concentrations were similar. This species difference in the brain:blood ratio of TRI is in general agreement with the richly perfused brain:blood partition coefficients estimated for rats (1.49) and mice (0.796) (Reitz *et al.*, 1987). Therefore, despite differences in the blood concentration of TRI, similar brain concentrations in mice and rats might be expected to result in similar effects on operant performance. The threshold concentration for response rate decreases in the present study is 35 $\mu\text{g/g}$ brain. However, mice exposed to 3500 ppm for 100 minutes exhibited no decline in VI-60 responding despite an end-exposure brain concentration of 132 $\mu\text{g/g}$. At 5000 ppm, a response rate decrease in mice was not evident until 20 minutes of exposure, when the brain concentration was $\geq 67 \mu\text{g/g}$. These results suggest that mice may be inherently less sensitive than rats to operant disruption by TRI while responding on a VI schedule.

In another investigation, Balster *et al.* (1982) reported a 25% reduction in fixed ratio responding of mice exposed to 2000 ppm TRI for 20 minutes. Based on results of the present study, a 25% decline in response rate would be expected to occur at a brain concentration of 60.8 $\mu\text{g/g}$. Interestingly, pharmacokinetic data (Holmberg *et al.*, 1977), once normalized for changes in exposure concentration, indicate that the brain concentration of TRI in mice after such an exposure would nearly equal the expected

value. However, since the schedule of reinforcement influences the sensitivity of operant behavior to toxicants, such a comparison may be somewhat misleading.

The effects of TRI on human behavior are also interesting in light of the present study. Mackay *et al.* (1987) exposed human volunteers to 175 and 350 ppm TRI for 3.5 hours, during which psychomotor tests were periodically administered and blood withdrawn for analysis. Deficits in tracking skill and reaction time were first observed at blood concentrations (\approx 0.50 and 1.60 $\mu\text{g}/\text{ml}$) well below the threshold blood level for response rate decreases in the present study (15 $\mu\text{g}/\text{ml}$), which can only be explained in small part by the differing richly perfused brain:blood partition coefficients reported for humans (3.40) and rats (1.49) (Reitz *et al.*, 1987). In addition, when steady-state blood levels measured in humans exposed to 350 ppm (Mackay *et al.*, 1987; Nolan *et al.*, 1984) are multiplied by the estimated richly perfused brain:blood partition coefficient, estimated brain concentrations range from 5.96-10.88 $\mu\text{g}/\text{g}$. These values are 17-31% of the threshold brain concentration necessary for response rate suppression in the rat, yet are sufficient to elicit psychomotor impairment in humans (Mackay *et al.*, 1987). Tracking skill and reaction time in humans therefore, appear to be far more sensitive to the effects of TRI than the well conditioned and highly motivated operant responding of rats. Stewart *et al.* (1961) have published results that suggest the same may be true for lightheadedness in humans.

Unfortunately, the present effort represents one of only a few attempts to correlate the pharmacokinetics of TRI with CNS pharmacodynamics. Indeed, few studies with any solvent, with the exception of alcohol, have had this goal in mind. Among these is a study by Riihimaki and Savolainen (1980) in which impaired body balance in volunteers was not only correlated with blood m-xylene concentrations, but was also shown to depend on a rapid rise of the blood m-xylene level. In addition, Bruckner and Peterson (1981) have shown that the magnitude of toluene-induced impairment of reflexes and unconditioned performance in mice is paralleled by blood and brain concentrations of

toluene. More recently, Kishi *et al.* (1993) have reported a relationship between blood trichloroethylene levels and shock avoidance performance decrements in rats. It is clear that due to ethical considerations, further progress in understanding the risk that solvents pose to the human CNS rests with performing animal studies. It is therefore imperative that the most sensitive behavioral tests be used in conjunction with physiologically and anatomically well characterized animals to create a data base to which state-of-the-art extrapolation methods can be applied.

The relationship between blood and brain concentrations of solvents and behavior is obviously complex. Such potential confounding factors as acute neuronal adaptation, biphasic response patterns, and uncertainty as to the neurological basis of many behaviors contribute to this complexity and will clearly make these relationships more difficult to determine. In addition, solvents have been shown to differentially distribute in the brain on the basis of regional lipid content (Gospe and Calaban, 1988; Ameno *et al.*, 1992) and, therefore, considering the CNS as a single homogeneous compartment for kinetic purposes or as a dose metric may be inappropriate. However, as pointed out by Weiss (1988), pharmacokinetic models deserve to be accompanied by behavioral endpoints of at least equal quantitative stature. Lack of knowledge of the relationship between blood or brain concentration and altered behavior would render such models inappropriate for interspecies extrapolation. Thus, the study described herein represents an initial effort to utilize behavioral modifications as an acceptable toxicological endpoint for risk assessment.

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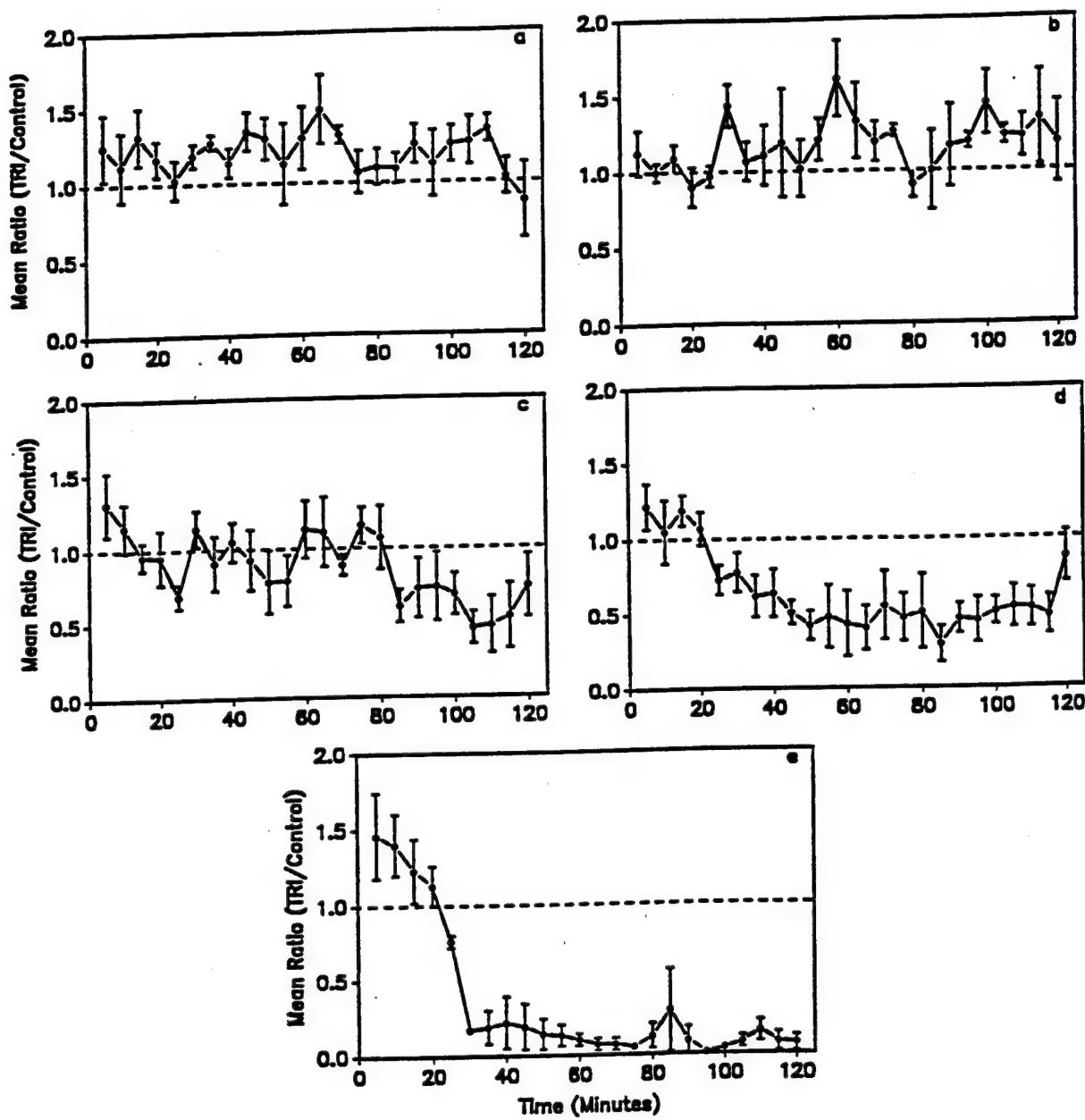


Figure 1: Operant responding during the 20 minutes preceding and 100 minutes following the onset of continuous exposure to a) 500, b) 1000, c) 2000, d) 3500 and e) 5000 ppm TRI. Each data point represents the mean operant response ratio (TRI/Control) \pm SE of five rats during each 5-minute interval of 120-minute operant sessions.

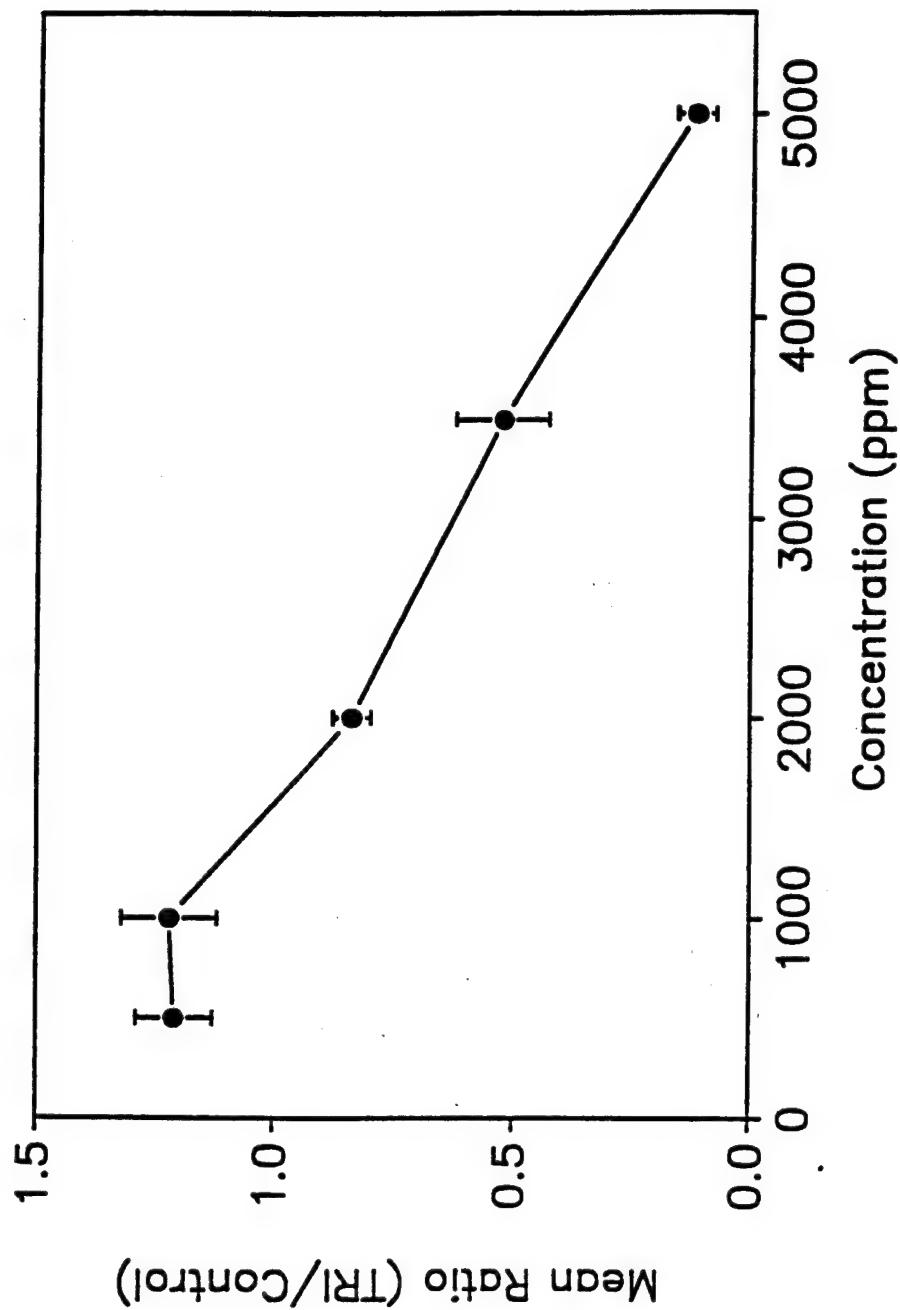


Figure 2: Dose-response curve relating operant responding to exposure concentration of TRI. Each data point represents the average \pm SE of mean operant response ratios (TRI/Control) during 100-minute exposures to 500, 1000, 2000, 3500 and 5000 ppm TRI.

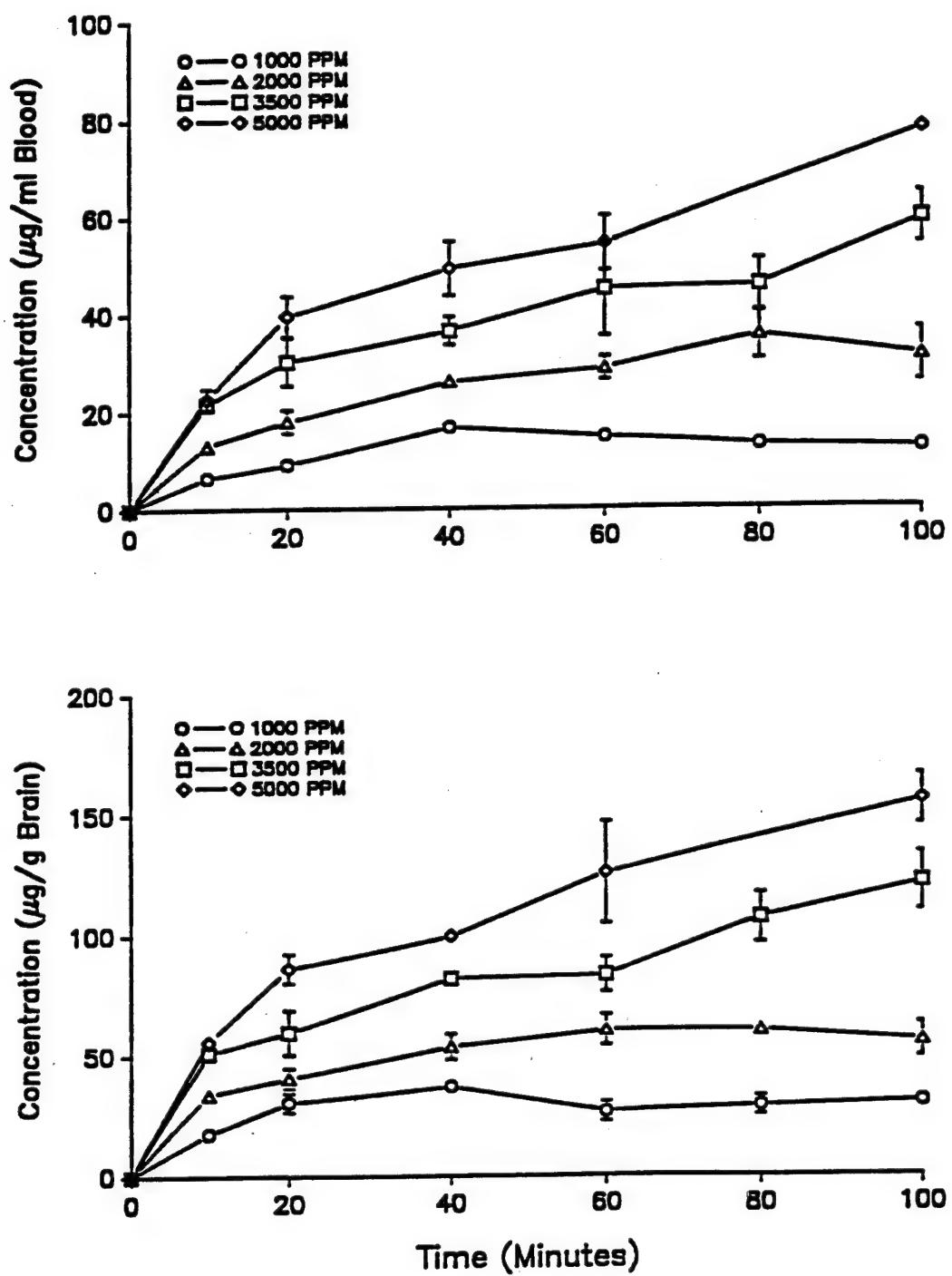


Figure 3: Uptake of TRI in a) blood and b) brain during 100-minute exposures to 1000, 2000, 3500 and 5000 ppm TRI. Each data point represents the mean \pm SE of five rats. SE bars are obscured by symbols in some cases.

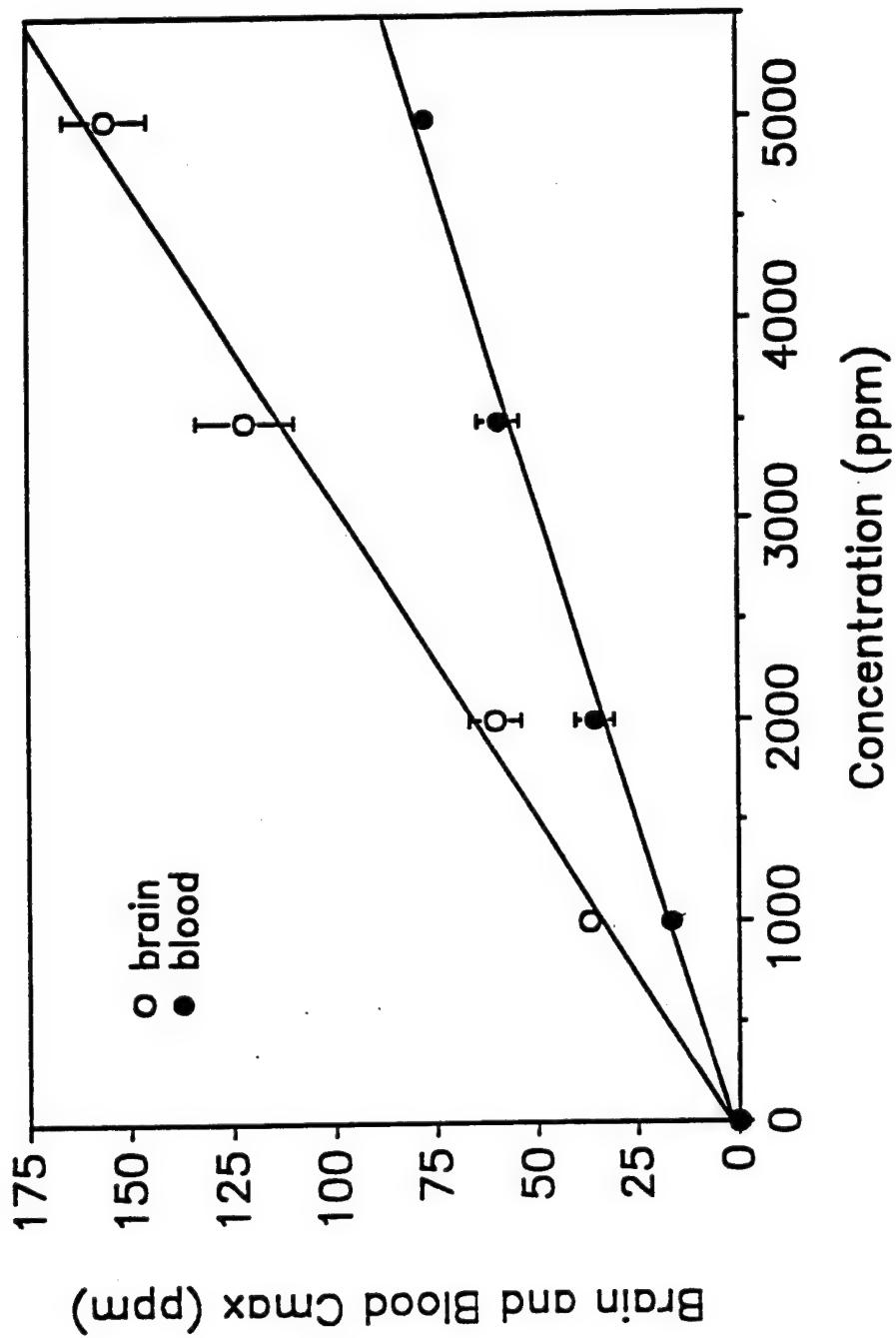


Figure 4: Maximum blood and brain concentrations during 100-minute exposures to TRI, as a function of inhaled concentration. Each data point represents the mean \pm SE of five rats.

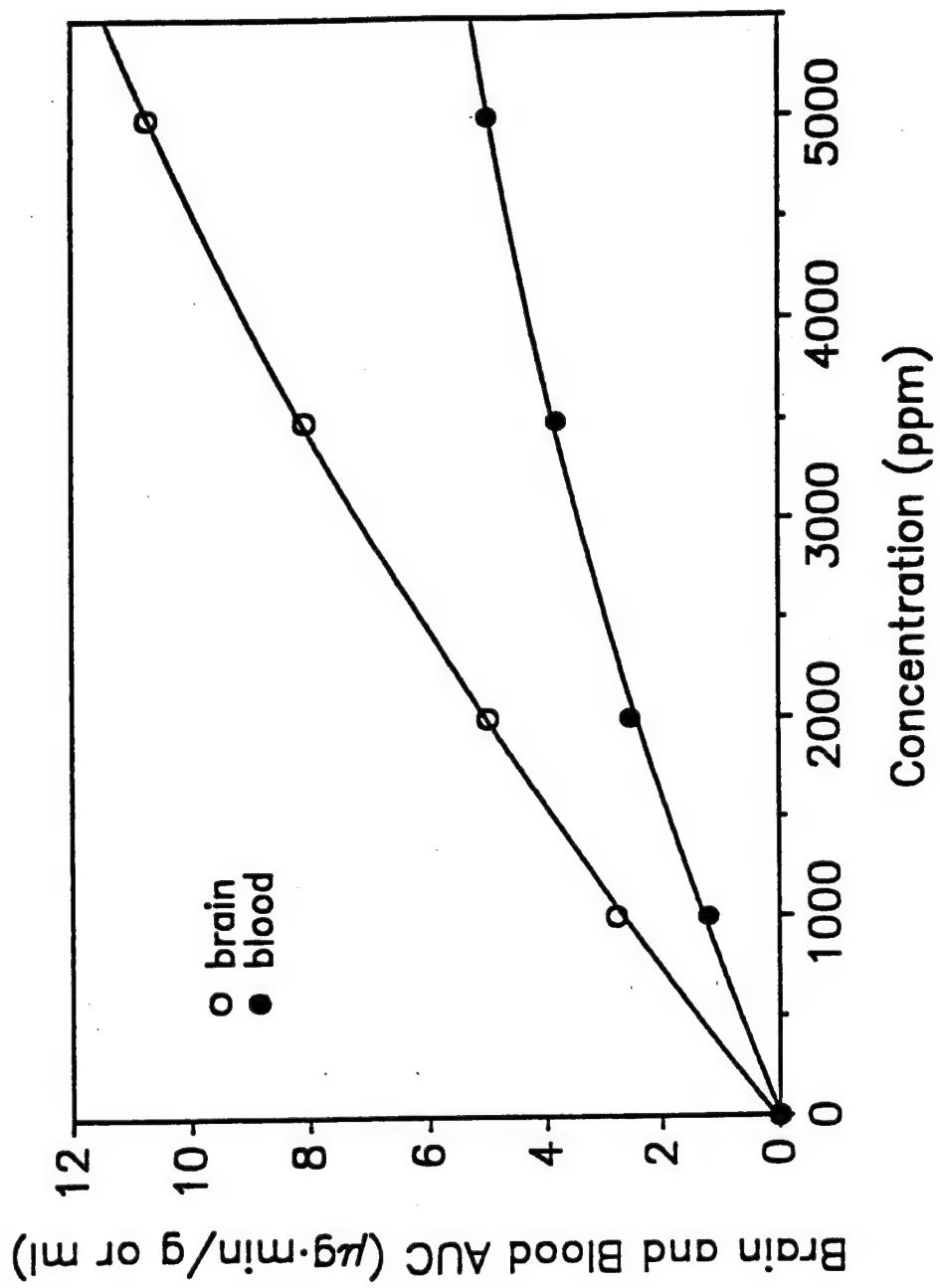


Figure 5: Areas-under-the-blood- and brain-concentration versus time curves presented in Figure 3, as a function of inhaled concentration.

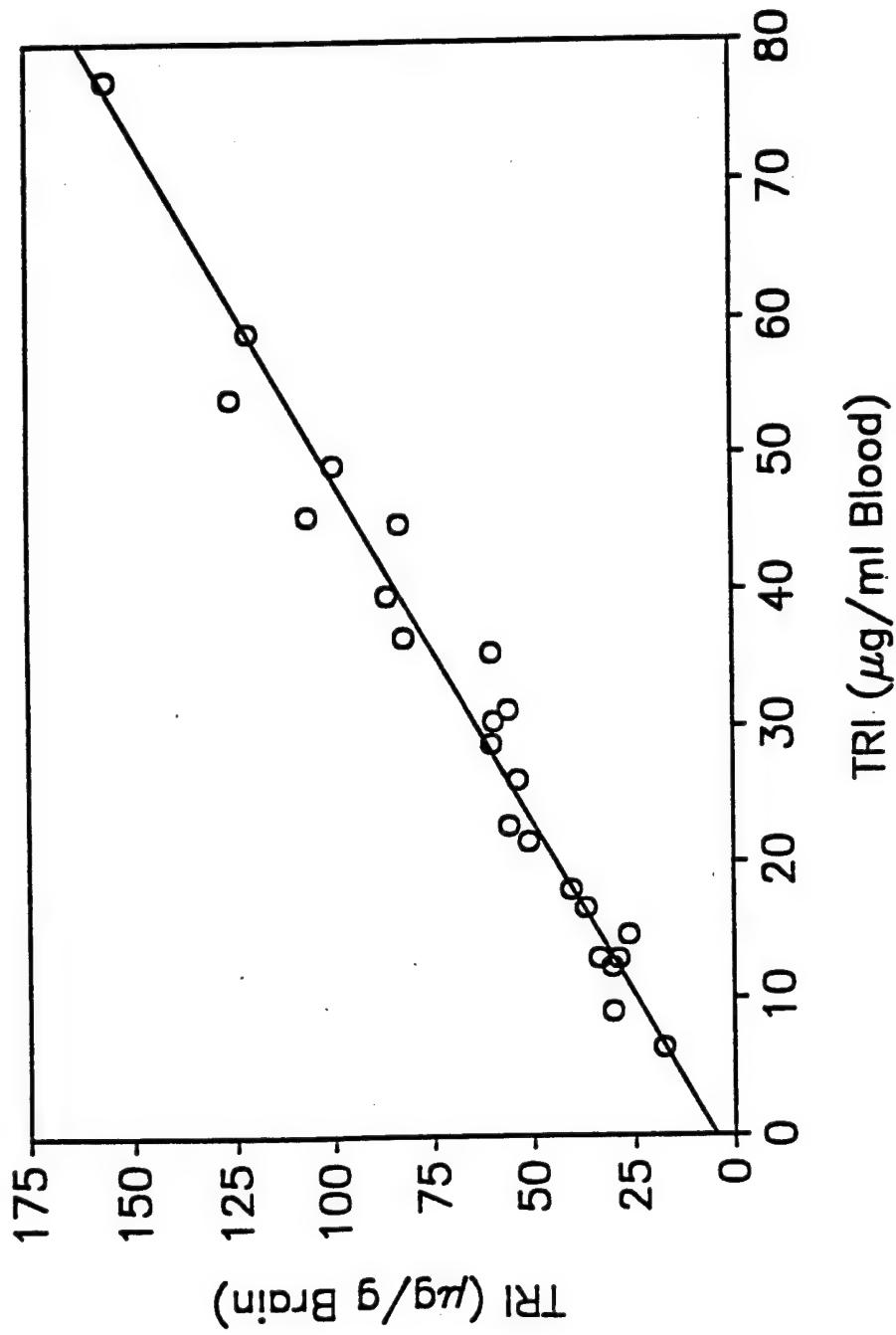


Figure 6: Scatter plot relating blood and brain concentrations of TRI. Each data point represents the mean blood and brain concentration of five rats after 10, 20, 40, 60, 80 or 100 minutes of exposure to 1000, 2000, 3500 or 5000 ppm TRI. The equation of the regression line is $y = 1.977x + 4.587$.

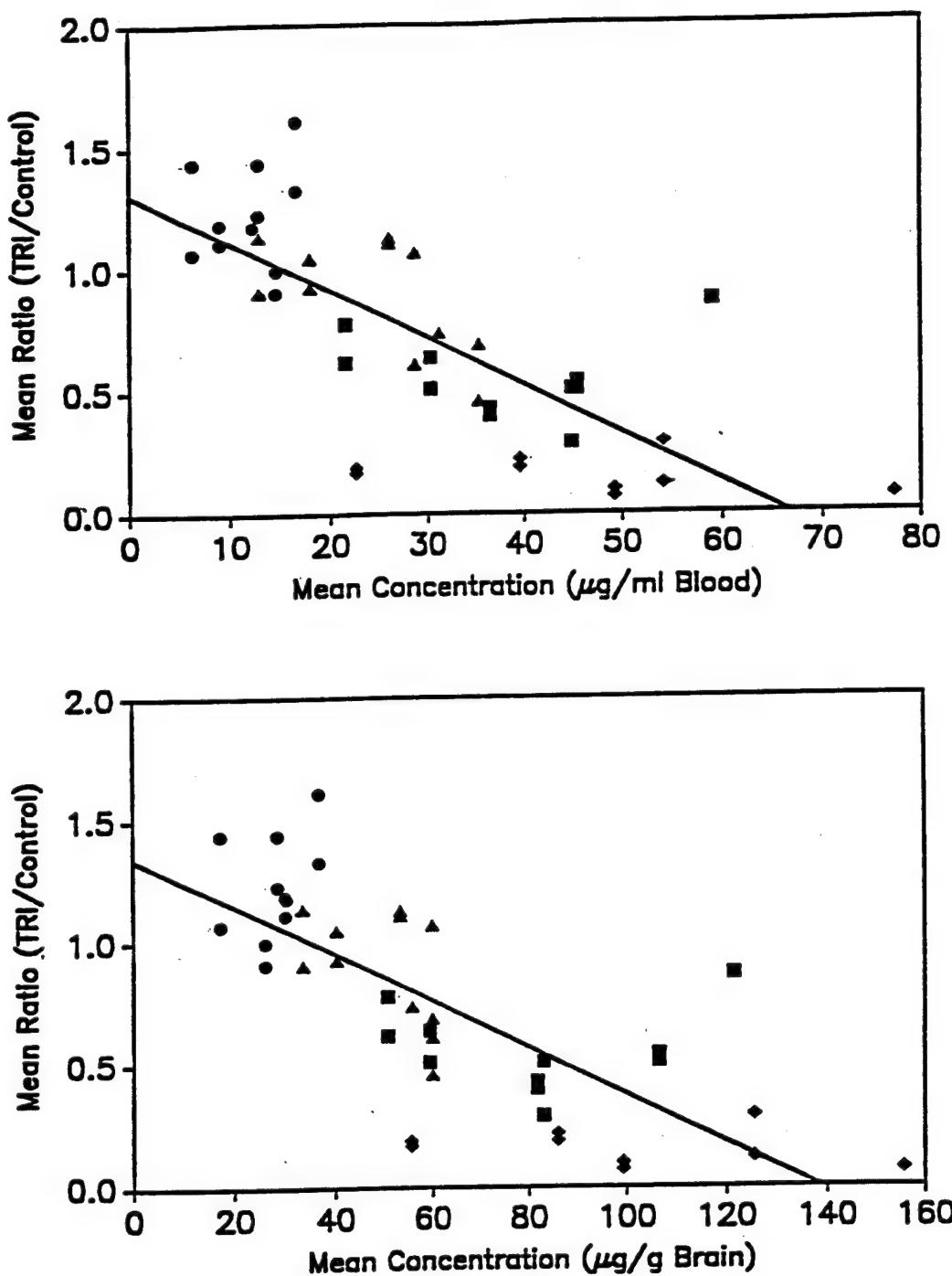


Figure 7: Scatter plots relating operant responding to a) blood and b) brain concentrations of TRI. Each data point represents the mean blood or brain concentration of five rats after 10, 20, 40, 60, 80 or 100 minutes of exposure to 1000 (●), 2000 (▲), 3500 (■) or 5000 ppm TRI (◆), as well as the mean operant response ratio (TRI/Control) of five rats during the 5-minute intervals immediately preceding and following blood and brain collection. The equations of the regression lines are $y = -0.019x + 1.29$ (blood) and $y = -0.0097x + 1.34$ (brain).

APPENDIX K

Warren, D.A., Reigle, T.G., and Dallas, C.E. "Dose-response curves for the effect of 1,1,1-trichloroethane on the operant behavior of singly and repeatedly exposed rats." To be submitted to *Journal of the American College of Toxicology*.

DOSE-RESPONSE CURVES FOR THE EFFECT OF 1,1,1-TRICHLOROETHANE
ON THE OPERANT BEHAVIOR OF SINGLY AND REPEATEDLY
EXPOSED RATS¹

¹Warren, D.A., Reigle, T.G., and Dallas, C.E. To be submitted to *Journal of the American College of Toxicology*.

ABSTRACT

A design feature of most dose-response studies involving schedule-controlled operant behavior is the repeated administration of different doses of the test substance to the same experimental animal. Repeated dosing raises the question of whether or not an animal's initial exposure to a chemical agent alters its behavioral response to subsequent exposures. To address this question, a dose-response curve for the effect of inhaled 1,1,1-trichloroethane (TRI) on the rate of lever-pressing for milk delivery was generated with repeatedly exposed rats (i.e., a within-subject design) and compared to dose-response data obtained from rats receiving a single inhalation exposure to TRI (i.e., a between-group design). Relative to that generated with singly exposed rats, the dose-response curve generated by repeated exposure was shifted to the left. This suggests that the behavioral effects of rate-decreasing concentrations of TRI are augmented by previous exposures. This residual effect is apparently not due to the accumulation of pharmacologically active substances or to the development of an aversion to responding, since TRI is rapidly eliminated following exposure and solvent-free responding was unaffected 24 hours post exposure. Instead, the results of this study support the well established belief that an animal's response to a drug or chemical agent can be modified by its prior behavioral and exposure history. Thus, comparisons of single and repeated exposures are essential for fully accurate interpretations of the behavioral consequences of solvent exposure.

INTRODUCTION

Inhalation of volatile organic solvents occurs in the workplace and as a form of drug abuse (Balster, 1987). Such solvents are capable of producing a general depression of the central nervous system (CNS), with consequent impairment of cognition and motor

skills in a concentration-dependent manner. In recent years, there has been an increased interest in the behavioral effects of these agents with a resultant increase in the study of their effects on schedule-controlled operant behavior (SCOB). Changes in operant behavior are thought to reflect effects of solvents in laboratory animals which are comparable to psychomotor changes in humans (ATSDR, 1994). As a result, several reports have described the rate-increasing and rate-decreasing effects of solvents on steady-state responding in rodents (Glowa, 1985; Evans and Balster, 1991). In some cases, the relationship between inhaled concentration and effect, i.e., the dose-response curve, has been determined. As behavior is increasingly being considered a potential regulatory endpoint, the need for dose-response determinations will likely increase in order to provide a basis for the development of rational limits for human exposure.

In view of the large number of solvents in use today and the continual introduction of new agents, rapid and inexpensive methods will be needed to assess their behavioral toxicity (Glowa *et al.*, 1983). Methods involving learned behaviors in general, and SCOB in particular, have often been deemed too time consuming and labor intensive for routine use (Wenger, 1990). However, unlike many of the more recently introduced behavioral tests, previous studies of SCOB have provided an extensive background of information against which the effects of solvents may be compared (Rice, 1988). In addition, simpler procedures such as the measurement of unconditioned reflexes and motor activity reflect different aspects of behavior and are often less sensitive measures of solvent exposure (Sette and Levine, 1986; Claudio, 1992). Therefore, the economical use of SCOB would be of considerable benefit to the study of behavioral toxicology.

One economical design feature common to most dose-response studies involving SCOB is the repeated administration of different doses to the same animal. Indeed, the practicality of SCOB largely depends on within-subject designs due primarily to the laborious task of training animals to some level of behavioral stability. In turn, the

appropriateness of some within-subject designs depends upon whether chemical exposure irreversibly changes an animal's behavior in such a manner that would be reflected in its behavioral reaction to subsequent exposures (Sidman, 1960). If irreversible changes occur, the resulting dose-response data would not be a pure function of concentration, but would also be a function of the consequences of previous exposure (Sidman, 1960).

The generation of cumulative dose-response curves by exposing animals to incrementally increasing solvent concentrations in a single operant session is a widely accepted practice in behavioral toxicology (Glowa *et al.*, 1983; Glowka, 1991; Glowka, 1993). With such a design, the potential for solvent accumulation is great, and exposure to early solvent concentrations might be expected to influence the behavioral reaction to subsequent exposures. Because solvents classically exhibit a rapid rate of elimination, an alternative means of generating dose-response curves using a within-subject design would be to allow sufficient time between exposures for complete solvent clearance. The question remains, however, whether or not solvent exposure in the absence of solvent accumulation, would also influence an animal's response to subsequent exposures. To address this question, dose-response curves for the effect of 1,1,1-trichloroethane (TRI) on the rate of lever-pressing for milk delivery were generated using both singly and repeatedly exposed rats. Similar dose-response curves would suggest that prior exposure has little or no influence on operant responding, whereas differing dose-response functions would suggest that previous TRI exposures exerted a residual effect. 1,1,1-Trichloroethane was selected for study because it has a short half-life, undergoes minimal metabolism, and has relatively well characterized effects on SCOB. Using this solvent, it was believed that the results of the current investigation would provide an enhanced understanding of the factors which influence animal behavior and would enable a more accurate interpretation of the behavioral consequences of solvent exposure.

MATERIALS AND METHODS

Chemical: 1,1,1-Trichloroethane of 97% + purity was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI).

Animals: Male Sprague-Dawley rats (275-350 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC). Rats were housed two per cage in suspended wire-bottom cages (36 x 20 x 20 cm) in a temperature- (22°C) and humidity- (45%) controlled room with a 12-hour light-dark cycle (light: 0700-1900 hr). Rats were acclimated for at least 7 days prior to use, during which time food (Purina Lab Chow #5001, Ralston Purina Co., St. Louis, MO) and tap water were available *ad libitum*. All experiments were conducted during the light phase of the light-dark cycle.

Behavioral and Exposure Apparatus: Operant sessions were conducted in a slotted test cage (Coulbourn Instruments, Inc., Lehigh Valley, PA) equipped with a house light, response lever, liquid delivery trough and dipper, and a stimulus light above the delivery trough that remained lit during the availability of the milk reinforcer. The test cage was interfaced via LabLinc (Coulbourn Instruments, Inc., Lehigh Valley, PA) with an IBM-compatible 386 computer running COSMOS software (Coulbourn Instruments, Inc., Lehigh Valley, PA) that applied the operant performance schedule and recorded the number of responses and reinforcers in each 5-minute interval of the operant session. The test cage was positioned inside a 1.0 M³ Rochester-type dynamic flow inhalation chamber that served not only to expose the animals, but also to isolate them from extraneous stimuli. Nitrogen was passed through a glass dispersion flask of liquid TRI from which solvent vapor entered the chamber's influent air stream. A heating mantle was placed around the dispersion flask to generate vapor concentrations \geq 2000 ppm. The flask was enclosed in a plexiglass safety box under constant negative pressure. Exhaust air from the inhalation chamber and safety box was vented through HEPA and activated charcoal filters prior to its release into the environment. Vapor concentrations

were continuously monitored with a Miran 1B2 portable infrared spectrophotometer ($\lambda = 9.4 \mu\text{m}$) (The Foxboro Co., East Bridgewater, MA) interfaced with a microcomputer-based Foxboro DL 332F Datalogger (Metrosomics Inc., Rochester, NY). The Miran was calibrated with a closed loop system (The Foxboro Co., East Bridgewater, MA) and the calibration accuracy verified just prior to each exposure with liquid TRI injections that volatilized to produce concentrations spanning the calibration range. Target vapor concentrations were reached within 2-5 minutes and thereafter exhibited $\pm 5\%$ random fluctuation. Occasional adjustments in nitrogen flow and heating mantle temperature were necessary to maintain target concentrations.

Operant Behavior: Rats were transferred to individual polypropylene cages (48 x 25 x 20 cm) with corn cob bedding and stainless steel wire lids following acclimation. The animals were food-restricted ($10 \pm 0.25 \text{ g/day}$) during a period in which they were trained to lever-press for the presentation of undiluted evaporated milk (0.08 ml for 7 seconds/reinforcement) on a variable interval-30 second (VI-30) reinforcement schedule (i.e., a lever-press produced milk every half minute on average). Initially, rats were manually reinforced in 30-minute sessions for coming near the lever or inadvertently touching it. Once rats learned to respond independently, the mean interval between reinforcer availability was gradually increased to 30 seconds and the session length extended to 120 minutes. Rats responded in daily 120-minute sessions spaced 24 hours apart until their response rates stabilized, a process requiring 15-20 days. The criterion for stable behavior was four successive sessions in which the number of responses/second varied by less than 15% from the 4-day mean rate and showed no significant time trend. Once rats met the stability criterion, their behavior was monitored during exposure to clean air for 20 minutes, followed by a single concentration of TRI vapor (500, 1000, 2000, 3500 or 5000 ppm) for 100 minutes. Five chemically-naive rats were exposed to each TRI concentration.

The same methodology was followed in the preparation of animals for repeated TRI exposure. However, during efforts to establish stable baseline response rates, several rats exhibited reduced rates of lever-pressing in the terminal half of their operant training sessions. Thus, the reinforcement schedule was shifted from a VI-30 to a VI-60 (a lever-press produced milk every minute on average) in order to reduce the reinforcement density. Once rats responding on a VI-60 schedule met the stability criterion ($n = 9$), four rats were repeatedly exposed to TRI in the order 1000, 5000, 3000, 4000 and 2000 ppm (Group A), and the remaining five rats in the order 4000, 1000, 5000, 3000 and 2000 ppm (Group B). Exposures were separated by 48 hours, and 24 hours after each exposure, rats responded for 120 minutes in a solvent-free atmosphere.

Data Analysis: The control behavioral response of each singly and repeatedly exposed animal was calculated as the average number of lever-presses during the last 100 minutes of the four operant sessions used to meet the stability criterion. Time trends in responding were examined by linear regression analysis (Wallenstein *et al.*, 1980). The number of responses during the last 100 minutes of the solvent-free operant sessions that were conducted 24 hours after each exposure were reported as a proportion of control. The effect of each TRI concentration in individual rats was determined by calculating the ratio of the rate of responding in the presence of TRI to the control rate of responding (TRI/Control). Dose-response data are presented as the mean \pm standard error (SE) of these individual determinations. Additionally, the operant response ratios of the nine repeatedly exposed animals were plotted in comparison to the dose-response curve generated with singly exposed animals. Response ratios at 3000 and 4000 ppm were estimated for singly exposed animals from a least squares linear regression line ($y = -0.000253x + 1.405$; $r^2 = 0.99$) that was based on effects observed at 1000, 2000, 3500 and 5000 ppm. Student's t-tests were used to compare dose-response data of singly and repeatedly exposed rats with a minimum level of significance of $p \leq 0.05$. The EC₅₀ value (concentration of TRI expected to decrease responding by 50%) was determined

for singly exposed animals by solving the equation for the least squares linear regression line where $y = 0.5$, and the confidence interval (CI) was defined using SAS (SAS Institute, Cary, NC). Measures of variation are standard deviations (SD) unless otherwise specified.

RESULTS

Rats responding under the variable interval schedule displayed a characteristic moderate, but steady rate of responding, with little or no pausing evident after each reinforcement (Weiss and Cory-Slechta, 1994). The control response rates of animals singly exposed to 500, 1000, 2000, 3500 and 5000 ppm TRI were 0.31 ± 0.15 , 0.31 ± 0.17 , 0.46 ± 0.10 , 0.36 ± 0.08 and 0.44 ± 0.11 responses/second, respectively. The mean (\pm SD) control response rate of repeatedly exposed animals was 0.43 ± 0.25 responses/second, with a range from 0.15 to 0.76 responses/second for individual animals. Therefore, the use of slightly different variable interval schedules (VI-30 versus VI-60) did not result in response rate differences sufficient to account for any differential response to TRI between repeatedly and singly exposed animals. Neither the singly nor repeatedly exposed animals exhibited a significant time trend in the rate of responding during control or solvent-free operant sessions. Repeated exposures to TRI did not appear to affect solvent-free responding, as response rates returned to control levels when examined 24 hours post exposure (Figure 1). Such recovery occurred following all conditions of TRI exposure, including concentrations which nearly caused the complete cessation of responding 24 hours earlier.

A comparison of the dose-response curves generated by exposing groups of rats to a single TRI concentration and individual rats to multiple TRI concentrations is shown in Figure 2. Response ratios of singly exposed animals averaged 1.20 ± 0.15 , 1.20 ± 0.17 , 0.83 ± 0.22 , 0.53 ± 0.14 and 0.15 ± 0.16 for the 500, 1000, 2000, 3500 and

5000 ppm groups, respectively. For repeatedly exposed animals, response ratios during TRI exposure to 1000, 2000, 3000, 4000 and 5000 ppm averaged 1.17 ± 0.13 , 0.45 ± 0.08 , 0.18 ± 0.04 , 0.45 ± 0.17 and 0.25 ± 0.07 , respectively. Relative to that generated with singly exposed animals, the dose-response curve generated with repeatedly exposed animals was shifted to the left, indicative of a greater response to equivalent concentrations of TRI. Whereas the EC₅₀ in singly exposed animals was 3577 ppm (95% CI = 2864-4253 ppm), average response rate reductions of 55 and 82% were produced in repeatedly exposed animals at 2000 and 3000 ppm, respectively. These drastic response rate reductions occurred immediately upon the initiation of exposure in the majority of animals.

Response ratios for animals singly exposed to 3000 and 4000 ppm were estimated to be 0.65 ± 0.06 and 0.39 ± 0.06 , respectively. Student's t-tests detected highly significant differences in the response ratios of singly and repeatedly exposed animals at 2000 ($t = 4.25$, $df = 12$, $p < 0.0005$) and 3000 ppm ($t = 9.63$, $df = 12$, $p < 0.0005$). At these two concentrations, all nine repeatedly exposed animals exhibited response ratios at or below the average response ratio measured or estimated in singly exposed animals (Figure 3). The majority of repeatedly exposed animals also had lower response ratios at 1000, 4000 and 5000 ppm, although interanimal variability was high. Whereas the dose-response curve reflected increasing effects with increasing dose for singly exposed animals, this was not the case for repeatedly exposed animals. On average, rats in Groups A and B showed little or no difference in their responses to 2000, 3000, 4000 and 5000 ppm.

The statistically significant differences in response ratios of singly and repeatedly exposed animals at 2000 and 3000 ppm may have been due in part to the order of exposure concentrations. The lowest average response ratio for repeatedly exposed animals was at 3000 ppm, which immediately followed a concentration (5000 ppm) that had drastic rate-decreasing effects in all nine animals just 48 hours earlier. In addition,

2000 ppm was the fifth and final concentration for all nine repeatedly exposed animals, and therefore, exposure to three rate-decreasing concentrations had already occurred within the last 8 days.

DISCUSSION

Although the CNS is highly sensitive to the effects of many solvents, few studies have examined the dose dependency of solvent effects on steady-state behavior under the control of operant schedules. Balster *et al.* (1982) and Moser *et al.* (1985) have, however, demonstrated concentration-dependent decreases in response rates during TRI exposure in mice trained on fixed-ratio 100 reinforcement schedules. Both of these studies used repeated or cumulative exposure regimens for the determination of dose-response relationships and, although comparisons between these investigations and the current study are limited by the use of different species, exposure parameters and reinforcement schedules, comparable EC₅₀ values were obtained. The two previous studies reported EC₅₀ values of 2836 ppm (95% CI = 2042-3631 ppm) and 2727 ppm (95% CI = 1622-4365 ppm), versus an EC₅₀ for singly exposed animals in the present study of 3577 ppm (95% CI = 2864-4253 ppm). However, results of the current investigation indicate that prior exposure to rate-decreasing concentrations of TRI augments the rate-decreasing effects of subsequent exposures, thereby altering the dose-response relationship. That VI-60 responding in the current study had recovered to control rates when measured 24 hours post exposure would indicate that such recovery is not synonymous with the complete reversibility of TRI's behavioral effects. Complete reversibility may only be necessary, however, when quantitative accuracy is a primary concern, as when such data are used in a risk assessment context. Indeed, the residual effect of rate-decreasing exposures may be beneficial for hazard identification purposes by increasing test sensitivity.

The residual effect of rate-decreasing TRI concentrations could be due to pharmacological factors, behavioral factors, or both (Liang *et al.*, 1983). That pharmacological factors are responsible is unlikely since there is little potential for TRI to accumulate during the exposure regimen employed in the current study. Schumann *et al.* (1982a) have reported, for example, that 96-99% of the total body burden of TRI in rats is eliminated 24 hours after a 6 hour inhalation exposure to 150 and 1500 ppm. Although higher concentrations were used in the present study, animals were only exposed for 100 minutes and should also be essentially free of TRI after 24 hours. Furthermore, rats exposed to 500 ppm for 6 hours/day for 4 days had only trace amounts of TRI in the brain and blood 17 hours after the end of the exposure period (Savolainen *et al.*, 1977). The observation that rapid elimination also occurs from the brain is significant, since the lipophilicity of TRI could potentially delay its mobilization from lipoidal tissues. Additional evidence also suggests that solvents do not exhibit delayed mobilization from the brain. Perchloroethylene and toluene, two solvents with greater fat:blood partition coefficients than TRI (Gargas *et al.*, 1989), have been shown to be eliminated from the brains of rats and mice at comparable or slightly faster rates than from the blood (Dallas *et al.*, 1994a; Benignus *et al.*, 1981; Bruckner and Peterson, 1981).

The ability of rate-decreasing TRI concentrations to augment the effects of subsequent exposures is also not likely to stem from the accumulation of metabolites, since metabolism plays only a minor role in TRI elimination. Rats have been reported to metabolize only 2-6% of the TRI absorbed during 6 hour inhalation exposures to 150 and 1500 ppm (Schumann *et al.*, 1982a, 1982b). Furthermore, repeated daily exposure of rats to 1500 ppm for approximately 16 months does not discernably alter the extent of metabolism, routes of excretion, or tissue concentration of TRI when compared to age-matched, singly exposed animals (Schumann *et al.*, 1982b). These findings indicate that the brain deposition and pharmacokinetic profile of TRI were comparable in the singly

and repeatedly exposed animals at equivalent exposure concentrations in the current study. That solvent-free responding was unaffected by repeated exposure to TRI in itself discounts the role of parent compound or metabolite accumulation in the residual effects of TRI on schedule-controlled responding. If TRI and its metabolites are present at the time of solvent-free responding, they must be so at pharmacologically inactive concentrations.

That an animal's response to solvent exposure can be influenced by its behavioral and exposure history has been previously suggested by Liang *et al.* (1983) who determined cumulative concentration-effect functions in mice on five successive days for carbon disulphide's (CS_2) effect on the interruption of a light beam maintained by a FI-60 schedule of milk presentation. Two years earlier, Glowa (1981) had reported the results of a very similar experiment with toluene. The rate-decreasing effects of CS_2 and toluene became progressively greater with repeated daily exposure, even though parent compound and metabolite accumulation was discounted as a possible cause. Another possible explanation for the residual effects of CS_2 and toluene, as well as for TRI in the present study, is that an aversion to responding developed as a result of pairing possibly irritant or toxic concentrations of the solvents with operant stimuli. While this was a viable explanation for why the repeated administration of the pesticide chlordimeform augments its behavioral toxicity and severely decreases control responding (Glowa, 1986), it too can be largely discounted on the basis that CS_2 , toluene and TRI exposure failed to affect solvent-free responding.

Although previous exposure to TRI clearly affects behavioral responsiveness to subsequent exposures, ample evidence exist that suggest that this is not a generalized phenomenon. For example, Glowa and Dews (1987) have shown that the cumulative dose-response curve for ethyl acetate on schedule-controlled responding in mice previously exposed to acetone is nearly identical to that obtained in exposure-naive mice. Likewise, the effects of toluene on schedule-controlled responding in mice previously

exposed to acetone, ethyl acetate, and methyl ethyl ketone were similar to those in mice exposed initially to toluene (Glowa and Dews, 1987). Furthermore, the concentration-effect curve for cumene on schedule-controlled responding in experimentally-naive mice is superimposable on the curve generated with mice previously exposed to other alkyl benzenes (Tegeris, 1991). However, since both current and previous results (Glowa *et al.*, 1983; Tegeris, 1991) indicate that repeated and cumulative dosing may result in a shift of the concentration-effect curve to the left of that generated with single exposures, care should be taken when designing and interpreting studies evaluating the dose-dependency of solvent-induced behavioral effects.

The comparison of dose-response curves as a method of investigation in behavioral toxicology is labor intensive, but has proven to be extremely informative in the current and previous studies (Glowa and Dews, 1983; Glowá *et al.*, 1983; Glowá, 1990; Tegeris, 1991). The results of the current investigation suggest that the residual effect observed may be due to the behavioral effect of TRI itself, that is, it may be more likely to occur following exposure to concentrations that decrease responding. These data support the well established belief that an animal's response to a drug or chemical can be modified by its prior behavioral and exposure history, even when such history is not reflected during exposure-free periods (Barrett, 1977; Glowá and Barrett, 1983).

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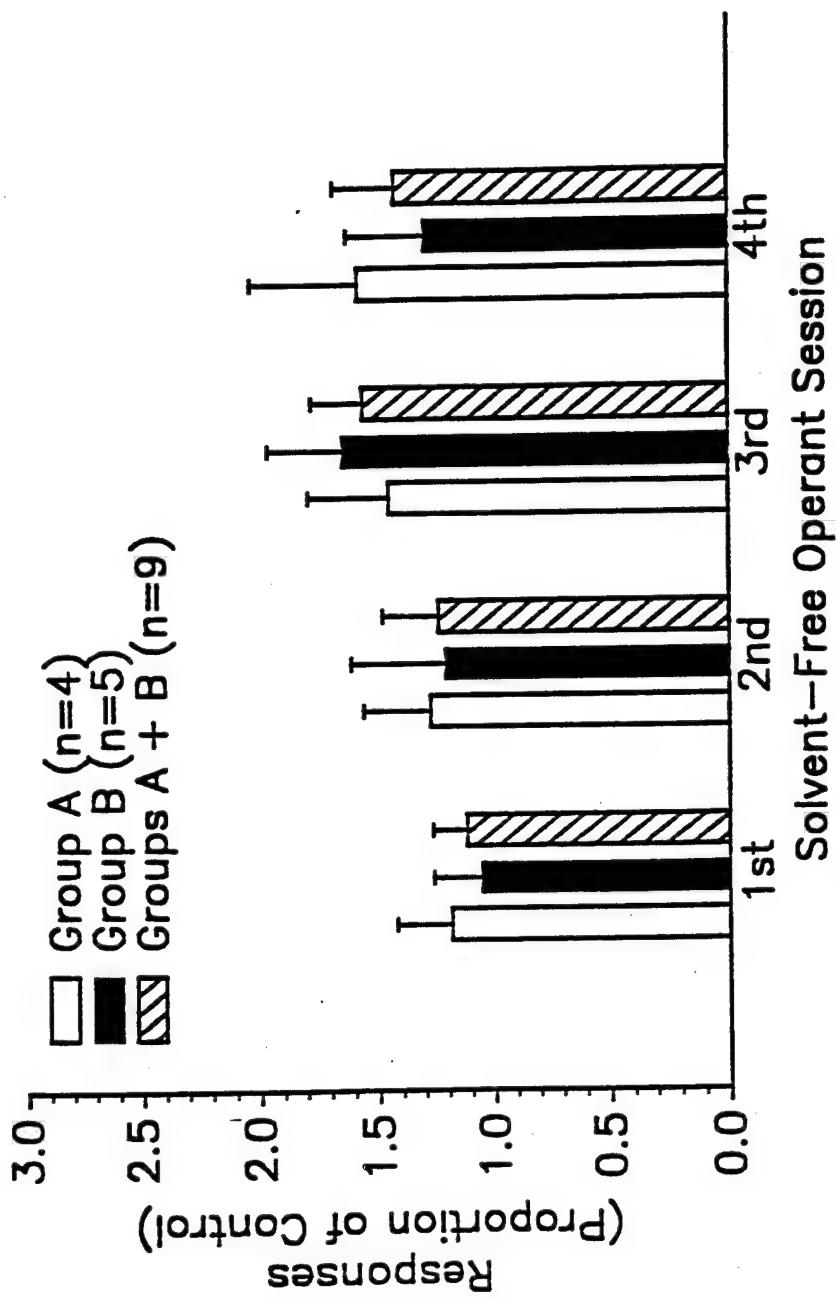


Figure 1: Number of responses during the last 100 minutes of solvent-free operant sessions that were conducted 24 hours after each exposure, reported as a proportion of control. Data are the mean \pm SE of repeatedly exposed rats in Group A, Group B, and Groups A + B.

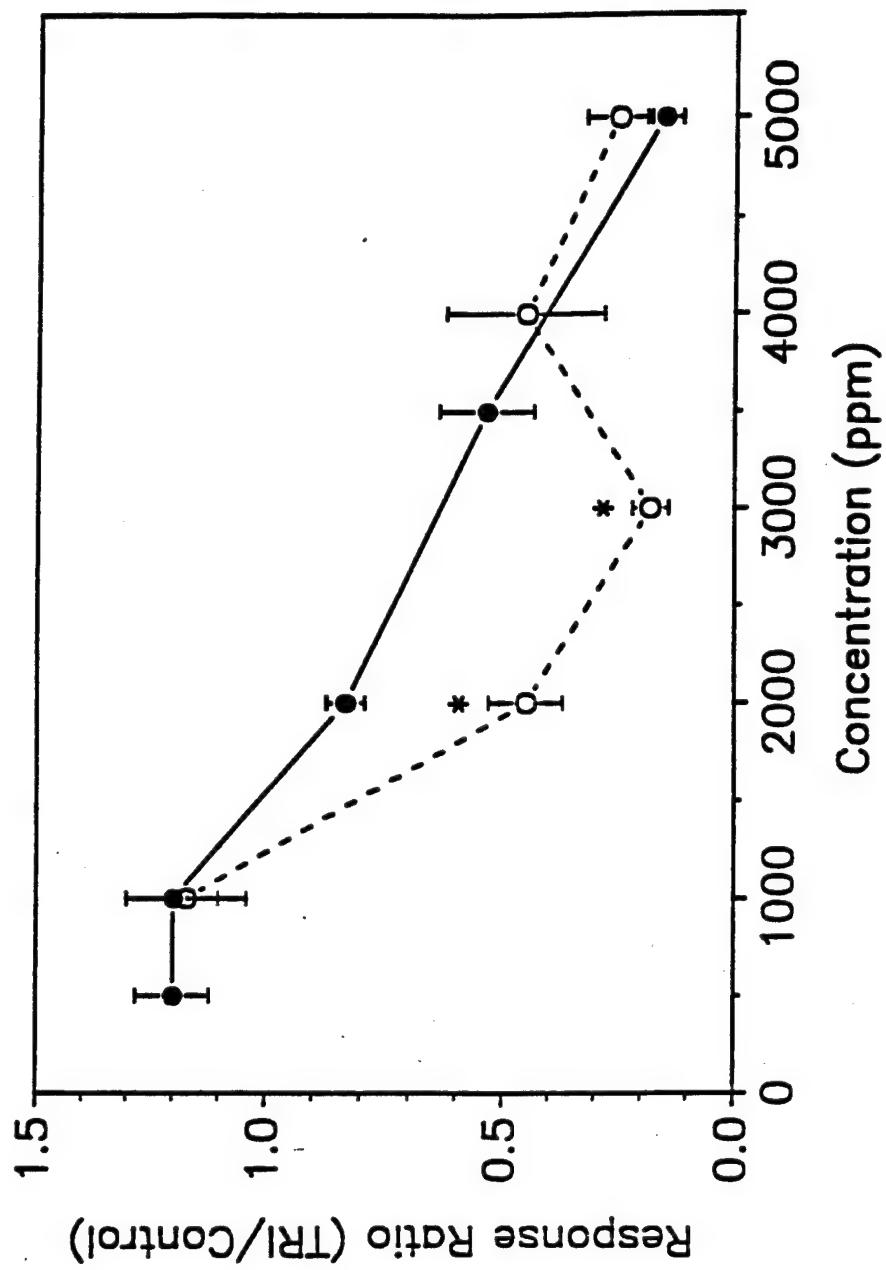


Figure 2: Dose-response curves for the effect of TRI on operant responding. Each closed circle (●) represents the mean operant response ratio \pm SE for five chemically-naive rats. Each open circle (○) represents the mean operant response ratio \pm SE for the nine rats repeatedly exposed to TRI. Asterisks signify concentrations at which the responses of singly and repeatedly exposed rats significantly differ ($p < 0.0005$).

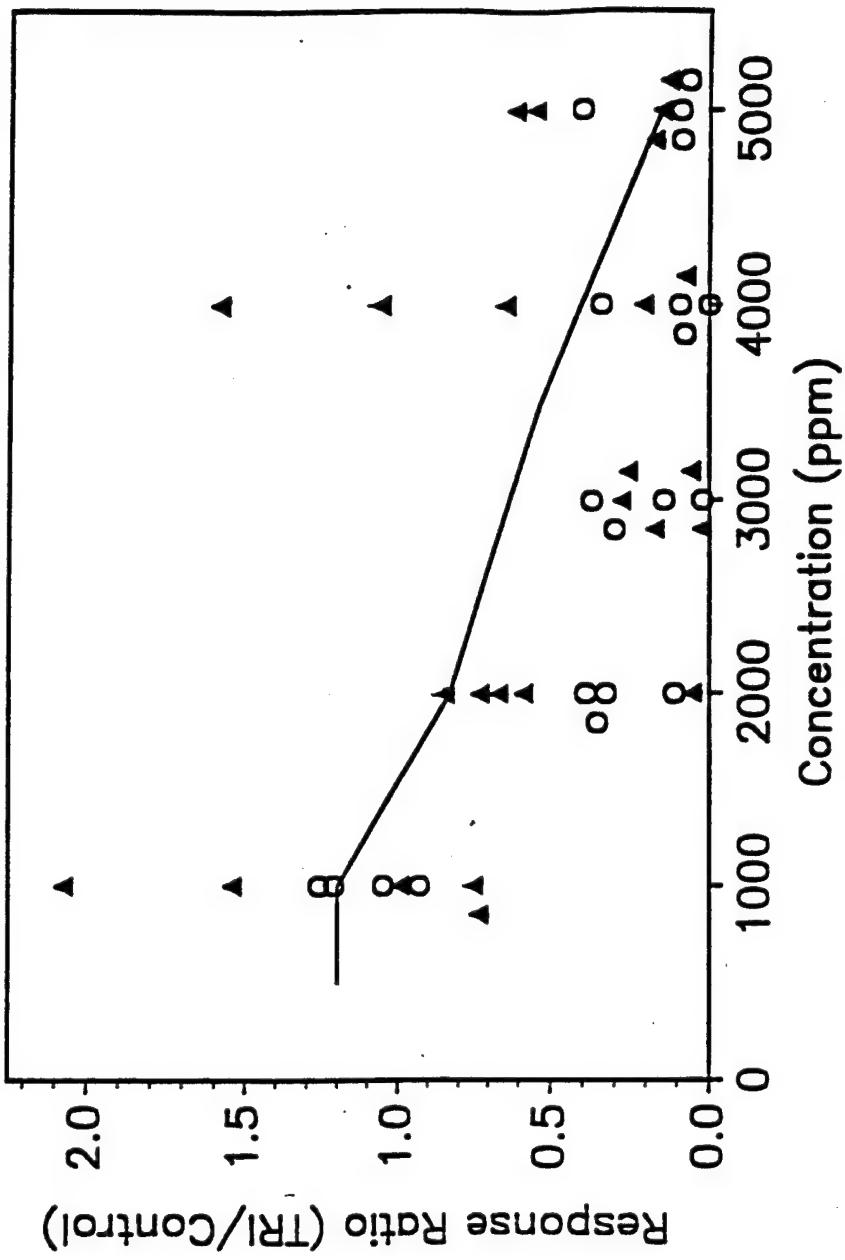


Figure 3: Operant response ratios of repeatedly exposed rats relative to a dose-response curve generated with singly exposed rats (solid line). Each open circle (○) represents one of four rats exposed in the order 1000, 5000, 3000, 4000 and 2000 ppm (Group A). Each closed triangle (▲) represents one of five rats exposed in the order 4000, 1000, 5000, 3000 and 2000 ppm (Group B). Symbols have been offset for clarity.

APPENDIX L

Warren, D.A., Bowen, S.E., Dallas, C.E., and Balster, R.L. "Biphasic effects of 1,1,1-trichloroethane on the locomotor activity of mice: Relationship to blood and brain solvent concentrations." To be submitted to *Neurotoxicology and Teratology*.

BIPHASIC EFFECTS OF 1,1,1-TRICHLOROETHANE ON THE LOCOMOTOR
ACTIVITY OF MICE: RELATIONSHIP TO BLOOD AND BRAIN
SOLVENT CONCENTRATIONS¹

¹Warren, D.A., Bowen, S.E., Dallas, C.E., and Balster, R.L. To be submitted
to *Neurotoxicology and Teratology*.

ABSTRACT

Despite the central nervous system (CNS) being a target of virtually all solvents, there have been few studies of solvent effects on unlearned animal behaviors. Little is known about the relationship of exposure concentration to behavioral effect, and quantitative data relating the toxicologically important target organ (i.e., brain) dose to behavioral effect are almost non-existent. To examine the relationships of blood and brain concentrations of 1,1,1-trichloroethane (TRI) to locomotor activity, mice were exposed to TRI (500-14,000 ppm) in static inhalation chambers for 30 minutes, during which locomotor activity was measured. Separate mice were exposed to the same concentrations for 6, 12, 18, 24 and 30 minutes to determine blood and brain concentration versus time profiles for TRI. The lowest TRI concentration studied (500 ppm) had no effect on activity, intermediate concentrations (1000-8000 ppm) increased activity immediately to levels that remained constant over time, and higher concentrations (10,000-14,000 ppm) produced biphasic effects, i.e., increases in activity followed by decreases. TRI concentrations in blood and brain approached steady-state equilibria very rapidly, demonstrated linear kinetics, and increased in direct proportion to one another. Locomotor activity increased monophasically (\approx 3.5 fold) as solvent concentrations increased from approximately 10-160 $\mu\text{g/g}$ brain and $\mu\text{g/ml}$ blood. As concentrations exceeded the upper limit of this range, the activity level declined and eventually fell below the control activity level at approximately 250 $\mu\text{g/g}$ brain and $\mu\text{g/ml}$ blood. The broad dose range employed demonstrated that TRI, like some classical CNS depressants, is capable of producing biphasic effects on behavior, supporting the hypothesis that selected solvents are members of the general class of CNS depressant drugs. By relating internal dose measures to locomotor activity, our understanding of the effects observed and their predictive value may be enhanced.

INTRODUCTION

The extent of solvent exposure by inhalation ranges from low concentrations produced by the proper use of commercial products to dangerously high concentrations encountered during recreational solvent abuse and accidents or misuse in industry (Moser and Balster, 1985). Following high-level exposure, a general depression of the central nervous system (CNS) may occur, with effects ranging from impaired cognition and motor skills to death. Acute solvent exposures to human volunteers have impaired performance in tests of manual dexterity, eye-hand coordination, perceptual speed and reaction time, and produced lightheadedness and imbalance (Torkelson *et al.*, 1958; Stewart *et al.*, 1961; Gamberale and Hultengren, 1973; Mackay *et al.*, 1987). Exposure limits have been established to protect workers from such effects (ACGIH, 1993), but the majority of these is based on a limited number of poorly documented responses to occupational exposures, often with little or no experimental confirmation from animal studies. In addition, few animal studies have evaluated the toxicologic properties of solvents under exposure conditions typical of solvent abuse (Bruckner and Peterson, 1981; Balster, 1987).

Several committees of the National Academy of Sciences have recommended examinations of learned and unlearned animal behaviors (as well as morphology) as the first steps in chemical hazard identification and evaluation (Buckholtz and Panem, 1986). One means of evaluating a chemical's effect on unlearned behavior is by measuring changes in locomotor activity. Activity measurements have several advantages, including ready quantification, automation, speed and limited cost. Nonetheless, few reports of the effects of solvents on locomotor activity are available (Wood and Colotla, 1990).

With the exception of the prototypical aromatic hydrocarbon, toluene, the effects of 1,1,1-trichloroethane (TCA) on animal behavior are better characterized than those of other industrial solvents. 1,1,1-Trichloroethane has produced rate changes in the food-

reinforced lever-pressing of mice (Balster *et al.*, 1982; Moser and Balster, 1986), altered performance on a match-to-sample discrimination task in baboons (Geller *et al.*, 1982), and impaired the ability of rats to avoid shock by lever-pressing (Mullin and Krivanek, 1982) and mice to remain atop an inverted screen (Moser and Balster, 1985). In addition, a single study has been published documenting the effects of TRI on motor activity (Kjellstrand *et al.*, 1985). Of the four inhalation concentrations examined in the study, only two produced activity changes in mice during exposure, making the study of limited value in defining the dose-response relationship.

As behavior is increasingly being considered a potential regulatory endpoint, the need for dose-response determinations will likely increase to provide a basis for the development of rational limits for human exposure. Because of interspecies differences in pharmacokinetics, the extrapolation of potential human risk from animal toxicity data without some knowledge of the corresponding target organ dose or a dose surrogate, may involve uncertainty. This uncertainty can be reduced by data that describe, for example, the blood and brain concentrations of a solvent and their relationships to corresponding levels of behavioral toxicity. Unfortunately, few studies have generated data of this type.

In the present investigation, blood and brain solvent concentrations and locomotor activity were measured in mice during exposure to nine concentrations of TRI vapor ranging from 500-14,000 ppm. This exposure regimen, extreme in terms of the number and range of concentrations, enabled the relationship between locomotor activity and inhaled solvent concentration to be well characterized. More importantly, it enabled the relationships between locomotor activity and blood and brain solvent concentrations to be described for the first time. By relating internal dose measures to locomotor activity, our understanding of the effects observed and their predictive value may be enhanced. In addition, the use of a broad dose range demonstrated that TRI, like many classical CNS depressants, produces biphasic effects on some behaviors, supporting the hypothesis

that selected solvents are members of the general class of CNS depressant drugs (Evans and Balster, 1991).

MATERIALS AND METHODS

Chemicals: 1,1,1-Trichloroethane of 97% + purity was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI) and Fisher Scientific (Fair Lawn, NJ). Burdick and Jackson Brand, High Purity Solvent isoctane was obtained from Baxter Healthcare Corp. (Muskegon, MI).

Animals: Male, albino Swiss-Webster (CFW[®]) mice (25-35 g) were obtained from Charles River Breeding Laboratories in Raleigh, NC and Wilmington, MA. Mice were housed singly in polypropylene cages with hardwood chip bedding (Sani-Chips, Montville, NJ) and stainless steel wire lids in temperature- (23°C) and humidity- (45%) controlled rooms with 12-hour light-dark cycles (light: 0700-1900 hr). Mice were acclimated for at least 7 days prior to use, during which time food (Agway Prolab RMH 3000, Syracuse, NY) and tap water were available *ad libitum*. Following acclimation, ten mice on which locomotor activity measures were made were food deprived and maintained at 80-85% of their free-feeding weight to emulate mice whose operant behavior was examined under identical exposure conditions in a separate study. The mice on which locomotor activity measures were made had previously been exposed to toluene (250-8,000 ppm). Mice used for blood and brain concentration determinations were not food restricted and were chemically naive.

Exposure Apparatus: Inhalation exposures were conducted in several identical static chambers which have been described previously (Moser and Balster, 1986). The chambers were 29-liter chromatography jars (Pyrex[®] 6942) with acrylic covers. A fan projected into each chamber above a wire basket which supported a piece of filter paper. A mouse was placed in the bottom of the chamber, the top put in place, and a calculated

amount of solvent injected onto the filter paper which volatilized with the aid of the fan to produce the desired vapor concentration. Vapor concentrations reached target levels in 1-3 minutes and remained stable for the duration of exposure. Concentrations within the chambers were continuously monitored with a Miran 1A infrared spectrometer (The Foxboro Co., East Bridgewater, MA) during locomotor activity measurements, and by gas chromatographic analyses of air samples serially extracted with a 1.0 ml gas-tight syringe and a 12" needle during blood and brain concentration determinations. The extracted air samples were directly injected into a Shimadzu GC-14A gas chromatograph (GC) equipped with an electron capture detector (ECD) and TRI concentrations were calculated from standard curves based on atmospheres of known concentration prepared in 9-liter glass jars. Chromatographic analyses were conducted using a stainless-steel column (182 x 0.317 cm) packed with 3% OV-17 (100-120 mesh) (Alltech Associates, Inc., Deerfield, IL). The GC operating conditions were injection port temperature, 150°C; column temperature, 80°C; ECD temperature, 360°C; flow rate for argon:methane (95:5) carrier gas, 60 ml/minute.

Locomotor Activity Measurements: Ten mice were adapted to the static exposure chambers for 30 minutes/day for 5 days, after which locomotor activity was measured for 30 minutes beginning immediately upon vapor generation. Mice were free to move about inside the chambers and locomotor activity was measured via two sets of photocells that bisected each chromatography jar. During the 30-minute exposure periods, locomotor activity was defined as the total number of photocell breaks or counts. Locomotor activity was recorded in 1-minute bins in order to maximize the sensitivity to detect temporal changes. Although rearing in some cases may have been detected as activity, the majority of activity counts were due to horizontal movement. Test sessions were conducted twice a week (Tuesdays and Fridays), with continued placement in the exposure chambers with air only on the remaining weekdays. Data from all Thursdays were averaged and served as the control performance. Mice were exposed to TRI

concentrations (500, 1000, 2000, 4000, 6000, 8000, 10,000, 12,000 and 14,000 ppm) in an ascending order and to only one concentration per test day. All experiments were conducted during the light phase of the light-dark cycle and at the same time each day for each animal.

Blood and Brain Sampling: Blood and brain solvent concentrations were determined in mice during exposure to the same concentrations at which locomotor activity was measured. Mice received one 30-minute chamber adaptation period on the day prior to solvent exposure. At 6, 12, 18, 24 and 30 minutes after the start of exposure, a mouse was removed from the exposure chamber and sacrificed by CO₂ asphyxiation. Blood (0.2-0.5 ml) was withdrawn from the inferior vena cava with a 1 ml tuberculin syringe and a 25 gauge needle and whole brains collected (\approx 0.4 g) within 1-2 minutes of sacrifice. Blood and brain samples were immediately placed into chilled scintillation vials containing 8 ml isoctane and 2 ml 0.9% saline. Four mice were sacrificed at each time point during exposure to each TRI concentration, except at 10,000 and 14,000 ppm only two mice were sacrificed at each time point.

Blood and Brain Analysis: Blood and brain samples were analyzed for TRI content by a method originally described by Chen *et al.* (1993). Briefly, blood and brain samples were homogenized as quickly as possible (5-10 seconds) with an Ultra-Turrax® homogenizer (Tekmar Co., Cincinnati, OH) to minimize volatilization of TRI, after which the samples were vortex-mixed for 30 seconds. The homogenates were then centrifuged at 2500 x g for 10 minutes at 4°C in the capped scintillation vials. An aliquot of the isoctane layer (5-10 μ l) was either transferred directly to an 8 ml headspace vial or first diluted with isoctane. The vials were capped immediately with Teflon®-lined latex rubber septa in aluminum seals and crimped tightly. Analyses of TRI were made with a Sigma Model 300 GC (Perkin-Elmer Co., Norwalk, CT) equipped with a HS6 headspace sampler (headspace sampler temperature, 70°C) and an ECD under the same chromatographic conditions previously described. 1,1,1-Trichloroethane

concentrations were calculated from daily prepared standard curves made by diluting various amounts of TRI in isoctane and corrected for the percent recovery characteristic of blood and brain samples. The percent recovery of TRI from blood (95.5%) and brain (94.3%) samples was previously determined by You *et al.* (1994) by injecting freshly harvested blood and brain samples with 4 μ l of a solution containing 1 mg TRI per 1 ml isoctane and analyzing the samples as previously described. The limit of detection for TRI was \approx 0.5 ng in 8 ml of air.

Data Analysis: Locomotor activity (mean \pm standard error (SE) of ten mice) during exposure to each TRI concentration was plotted relative to control activity. One-way analysis of variance was used to compare control activity to activity during TRI exposures of questionable effect (i.e., 500, 1000 and 2000 ppm). Areas under the blood and brain concentration versus time curves (AUC) were calculated by the trapezoidal rule (Rowland and Tozer, 1980), and maximum blood and brain concentrations (C_{max}) were obtained by visual inspection of data. Pooling data from the nine exposure concentrations, mean blood and brain solvent concentrations at 6, 12, 18, 24 and 30 minutes were plotted against each other, and the resulting scatter plot was subjected to least squares linear regression analysis and the degree of correlation measured by comparing the correlation coefficient to values in a t-distribution table (Gad and Weil, 1986). Pooling data once again from the nine exposure concentrations, mean blood and brain solvent concentrations at 6, 12, 18, 24 and 30 minutes were also plotted against locomotor activity (number of counts during exposure minus the number of counts during control) at corresponding times and exposure concentrations. The minimum level of significance was set at $p \leq 0.05$ for all statistical tests.

RESULTS

Locomotor activity during exposure to each of the nine TRI concentrations is shown relative to control activity in Figure 1. The results clearly demonstrate that inhalation of TRI produces concentration dependent biphasic stimulant and depressant effects on the locomotor activity of mice. Locomotor activity during exposure to 500 ppm did not differ from control activity ($F(1,58) = 0.497, p = 0.484$), which remained at a constant level for 30 minutes. Exposure to 1000 ppm did increase activity ($F(1,58) = 16.3, p = 0.002$), as did all concentrations in excess of this lowest-observed-effect-level. Exposures from 1000-8000 ppm elevated activity in the first minute of exposure and this increased activity was sustained at roughly the same level for the remainder of the exposure. Higher concentrations (10,000, 12,000 and 14,000 ppm) had a biphasic effect on locomotor activity. At these concentrations, activity initially increased monophasically with the time to peak activity level being dependent upon the inhaled concentration. With continued exposure, activity decreased at a rate that was also concentration dependent, and fell below the control level during the latter part of the exposure to 14,000 ppm. Only at 12,000 and 14,000 ppm did any mice cease activity continuously for greater than 1 minute. Ataxia was occasionally observed at the two highest concentrations. No seizures or fatalities occurred in any of the exposed animals.

The concentrations of TRI in blood and brain as a function of the degree and duration of exposure are shown in Table 1, and the C_{max} and AUC values based on these data are shown in Table 2. As expected from the abrupt changes in locomotor activity upon the initiation of exposure, TRI was rapidly absorbed from the lung as evidenced by its substantial presence in blood and brain after just 6 minutes of exposure. 1,1,1-Trichloroethane concentrations in blood and brain approached steady-state equilibria very rapidly, as blood and brain concentrations at 6 minutes averaged 77% of those at 30 minutes, and those at 12 minutes rarely differed from those at 30 minutes. With a few

isolated exceptions, TRI demonstrated linear kinetics over the broad dose range as blood and brain concentrations and AUC and C_{max} values were roughly proportional to the inhaled TRI concentrations.

As expected for a well-perfused and lipid-rich organ such as the brain, its pattern of TRI accumulation was very similar to that of the blood. The scatter plot relating mean blood and brain solvent concentrations (Figure 2) clearly illustrates that brain levels of TRI increase in direct proportion to blood levels ($\approx 1:1$), making blood and brain solvent concentrations highly correlated ($r = 0.97$, $df = 43$, $t = 24.89$, $p < 0.001$). These findings strongly suggest that blood and brain TRI concentrations are equally suited to relate to locomotor activity.

The scatter plots relating mean blood and brain solvent concentrations to locomotor activity (Figures 3 and 4) illustrate a monophasic increase in activity as solvent concentrations increase to approximately 160 $\mu\text{g/g}$ brain and $\mu\text{g/ml}$ blood. At higher solvent concentrations, activity decreases before eventually falling below the control level at approximately 250 $\mu\text{g/g}$ brain and $\mu\text{g/ml}$ blood. Based on the no-observed-effect-level of 500 ppm and the lowest-observed-effect-level of 1000 ppm, the estimated threshold concentration for locomotor activity effects is 10-20 $\mu\text{g/g}$ brain and $\mu\text{g/ml}$ blood.

DISCUSSION

Consistent with that shown for inhaled toluene and ip-administered ethanol (Hinman, 1987; Wood and Colotla, 1990; Middaugh *et al.*, 1992), the results of this study indicate that inhaled TRI produces concentration dependent biphasic effects on locomotor activity. The lowest concentration studied (500 ppm) had no effect on activity, intermediate concentrations (1000-8000 ppm) increased activity immediately to levels that remained constant over time, and higher concentrations (10,000-14,000 ppm) produced biphasic effects, i.e., increases in activity followed by decreases. In the only

other published study of TRI's effect on motor activity, groups of five mice were repeatedly exposed to either TRI (890, 1300, 2000 and 4000 ppm), methylene chloride, perchloroethylene, toluene or trichloroethylene for 1 hour (Kjellstrand *et al.*, 1985). The authors described the activity pattern for TRI as "...simple, consisting of an increase and a decrease closely related to the increase and decrease of the chamber concentration." They also noted that TRI was less effective at increasing activity than the other solvents, as it was effective only at the two highest concentrations tested. In an unpublished study, Albee *et al.* (1990) also observed an increase in the motor activity of rats exposed to 4000 ppm TRI for 6 hours per day for 4 days (ATSDR, 1994). Additional evidence of a biphasic effect for TRI comes from two studies of schedule-controlled operant behavior in which both increases and decreases in the rate of lever-pressing for milk delivery were observed during and following inhalation exposure (Moser and Balster, 1986; Warren *et al.*, 1993).

Biphasic effects have also been documented in humans for TRI, as well as for other volatile, lipophilic agents such as ethyl alcohol, halothane and xylene (Kalant, 1978; Roth, 1979; Seppalainen *et al.*, 1981). Body sway was decreased and reaction time improved in volunteers exposed to 200 ppm TRI, while both deteriorated at 400 ppm (Arlien-Soborg, 1992). 1,1,1-Trichloroethane's biphasic effect on human equilibrium has also been reported elsewhere (Savolainen *et al.*, 1982). In addition, exposure to 175 and 350 ppm TRI actually enhanced performance of volunteers in a test of distractability of attention and concentration (Mackay *et al.*, 1987).

Since most biphasic effects in humans have been on the vestibular system or neurophysiological parameters such as electroencephalograms and evoked potentials, there is little doubt that they are related to the accumulation of agents in the CNS. Many behavioral effects in laboratory animals, however, while generally considered to indicate specific neurological effects, could be produced by actions on other organ systems or the sensory-stimulative properties of the agents themselves (Tilson and Mitchell, 1984).

Recognizing this, Kjellstrand *et al.* (1985) exposed mice to an intense odorant stimulus (cologne) that was without effect on the locomotor activity of mice, indicating that solvent effects on activity were unlikely due to odor. This is further supported by observations of increased activity when toluene and ethanol are administered by a route other than inhalation (Wood and Colotla, 1990; Middaugh *et al.*, 1992). Since the lowest-observed-adverse-effect-level for acute TRI exposure in any rodent organ system other than the CNS is 8000 ppm (ATSDR, 1994), it is also unlikely that effects on other organ systems are responsible for TRI-induced activity changes. Additionally, solvent-induced toxicities other than the classical behavioral syndrome are usually metabolite mediated, and TRI is metabolized to a very limited degree (Schumann *et al.*, 1982a; 1982b). Moreover, activity increases, as opposed to decreases, are difficult to interpret as the mere disruption of activity by a nonspecific stressor such as irritation. Rather, activity increases would appear to reflect a biologically relevant event in the CNS, such as activation of the hypothalamo-pituitary-adrenal axis or the release of higher center control (Glowa, 1990). Therefore, we believe the biphasic effect observed in the present study was dependent on the deposition of TRI in the brain.

Hinman (1987) reported that toluene's biphasic effect on locomotor activity was consistent with the hypothesis that such behavior is dependent on the level of toluene in the CNS. He concluded that locomotor activity is increased at low CNS levels, while at higher CNS levels the hyperactivity is attenuated. Since Hinman's conclusion was based on a comparison of his locomotor activity data to time-courses of blood and brain concentrations determined by other investigators (Bruckner and Peterson, 1981; Benignus *et al.*, 1981), no attempt was made to quantitatively relate internal solvent concentrations to locomotor activity. On the basis of the present study, however, we can conclude that locomotor activity counts increase roughly 3.5 fold as TRI concentrations increase from approximately 10-160 µg/g brain and µg/ml blood. As concentrations exceed the upper limit of this range, activity levels decline until they reach control level at approximately

250 $\mu\text{g/g}$ brain and $\mu\text{g/ml}$ blood. As expected for a well perfused organ with a tissue:blood partition coefficient near unity (0.80) (Reitz *et al.*, 1987), brain TRI concentrations seemingly paralleled those of the blood. As a result, blood TRI concentrations also appear to be a reliable index of the locomotor activity level, agreeing with similar conclusions made in correlative studies of other behaviors with toluene and TRI (Bruckner and Peterson, 1981; Warren *et al.*, 1993).

Regardless of the behavior being examined, few studies have attempted to relate solvent pharmacokinetics to pharmacodynamics. In two such studies, blood and brain toluene concentrations in mice were highly correlated with performance in tests of reflexes and unconditioned behaviors (Bruckner and Peterson, 1981), as were blood and brain TRI concentrations in rats to the rate of schedule-controlled operant responding for milk delivery (Warren *et al.*, 1993). Also, Kishi *et al.* (1993) have reported a relationship between blood 1,1,2-trichloroethylene levels and shock avoidance performance decrements in rats. In studies of controlled human exposures, blood levels of m-xylene and TRI were measured and related to impaired body balance, eye tracking deficits and altered reaction times (Riihimaki and Savolainen, 1980; Mackay *et al.*, 1987). The present study is thought to be the first to relate internal dose measures of an industrial solvent to locomotor activity.

Given the diversity of neural influences on locomotor activity and the ubiquitous distribution of solvents within the brain (Rafales, 1986; Gospe and Calaban, 1988; Ameno *et al.*, 1992), studies of solvent effects on locomotor activity usually do not allow for a determination of mechanisms within the CNS. The value of locomotor activity may instead lie in its economy and sensitivity to solvent-induced changes. For example, the lowest behaviorally-active toluene concentration to date in animals increases locomotor activity (Wood and Colotla, 1990), and the lowest-observed-effect-level in the present study is about one-half that necessary to produce changes in schedule-controlled operant behavior under some reinforcement schedules (Balster *et al.*, 1982; Moser and Balster,

1986). Therefore, as was demonstrated in the present study, locomotor activity may be useful for the routine determination of target tissue dose-response relationships that will enable behavioral modifications observed in animals to be extrapolated to humans with a greater degree of certainty.

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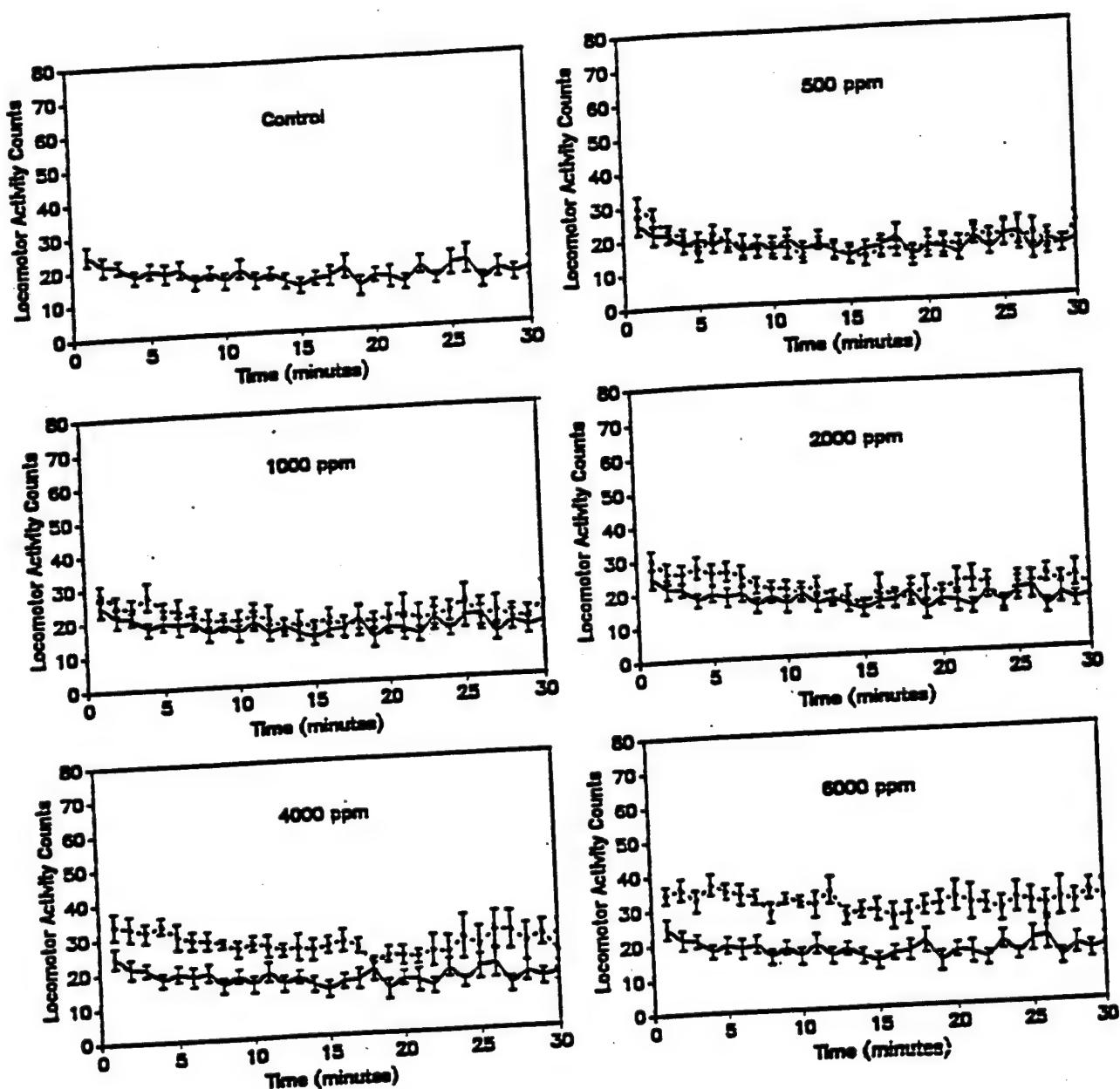


Figure 1: Locomotor activity during exposure to each of nine TRI concentrations (dashed lines) relative to control activity (solid lines). Each data point represents the mean \pm SE of ten mice.

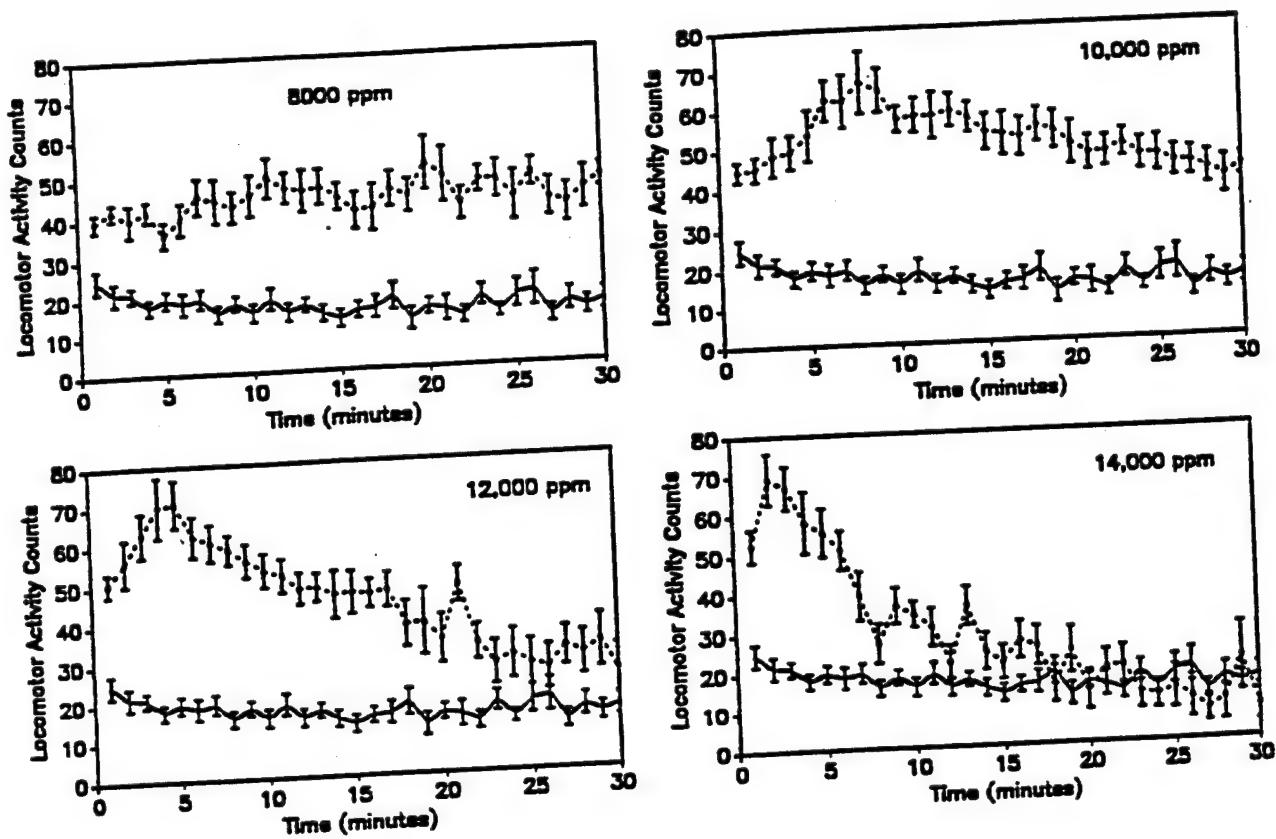


Figure 1 (Continued): Locomotor activity during exposure to each of nine TRI concentrations (dashed lines) relative to control activity (solid lines). Each data point represents the mean \pm SE of ten mice.

Table 1. BLOOD AND BRAIN CONCENTRATIONS OF TRI DURING INHALATION EXPOSURE

Exposure Concentration (ppm)	Time (minutes)			
	6	12	18	24
500	8.8 ± 2.3	9.8 ± 0.8	10.7 ± 0.8	10.9 ± 0.8
	6.6 ± 0.4	9.0 ± 0.4	9.6 ± 0.1	9.5 ± 0.8
1,000	17.5 ± 0.8	17.5 ± 1.3	20.4 ± 1.4	22.6 ± 0.9
	14.6 ± 0.7	13.4 ± 2.0	18.7 ± 0.7	18.1 ± 0.8
2,000	25.8 ± 1.6	32.2 ± 2.9	35.4 ± 2.0	34.7 ± 1.2
	25.3 ± 0.8	35.1 ± 3.6	38.0 ± 3.9	40.3 ± 2.6
4,000	52.6 ± 2.7	49.7 ± 8.4	52.3 ± 9.1	52.3 ± 5.4
	49.4 ± 2.3	56.6 ± 9.3	63.4 ± 4.1	60.9 ± 4.5
6,000	60.5 ± 4.3	88.3 ± 2.9	97.6 ± 5.8	81.1 ± 8.6
	61.8 ± 3.4	85.8 ± 4.1	88.8 ± 2.0	94.2 ± 5.3
8,000	109.7 ± 2.6	124.5 ± 9.8	120.8 ± 7.1	122.1 ± 6.1
	146.8 ± 11.1	144.7 ± 6.0	150.2 ± 4.7	146.8 ± 20.3
10,000	137.8 ± 47.4	161.8 ± 6.7	165.3 ± 14.7	195.8 ± 20.9
	121.9 ± 3.1	185.3 ± 6.1	168.5 ± 12.1	196.1 ± 30.7
12,000	156.0 ± 0.5	237.0 ± 23.0	224.5 ± 62.0	214.0 ± 10.0
	195.5 ± 21.7	185.0 ± 6.2	211.2 ± 11.7	217.7 ± 6.8
14,000	195.6 ± 11.5	239.3 ± 4.6	250.8 ± 13.8	256.5 ± 14.9
	146.1 ± 9.6	202.3 ± 15.0	194.3 ± 1.1	304.7 ± 41.4

Values are the mean ± SE of four mice, except at 10,000 and 14,000 ppm values are the mean ± SB of two mice.
 Brain concentrations of TRI are presented beneath blood concentrations.

Table 2. PHARMACOKINETIC PARAMETERS DURING INHALATION EXPOSURE

Exposure Concentration (ppm)	AUC ($\mu\text{g} \cdot \text{min}/\text{ml}$) (blood)	AUC ($\mu\text{g} \cdot \text{min}/\text{g}$) (brain)	C_{\max} ($\mu\text{g}/\text{ml}$) (blood)	C_{\max} ($\mu\text{g}/\text{g}$) (brain)
500	271.9	234.7	10.9 \pm 0.8	9.6 \pm 0.1
1,000	526.8	437.5	22.6 \pm 0.9	18.7 \pm 0.7
2,000	879.7	930.6	37.2 \pm 3.3	40.3 \pm 2.6
4,000	1441.9	1489.5	67.2 \pm 2.2	69.3 \pm 5.4
6,000	2253.5	2272.1	97.6 \pm 5.8	94.2 \pm 5.3
8,000	3277.2	3992.0	138.1 \pm 3.7	153.4 \pm 4.4
10,000	4578.1	4554.8	204.6 \pm 10.9	196.0 \pm 30.7
12,000	5664.9	5578.1	237.0 \pm 23.0	240.8 \pm 10.9
14,000	6425.9	5862.2	257.7 \pm 6.9	304.7 \pm 41.4

Values are the mean \pm SE of four mice, except at 10,000 and 14,000 ppm values are the mean \pm SE of two mice.
 AUC is the area under the curve that describes the concentration of TRI in blood and brain from 0-30 minutes.
 C_{\max} is the maximum concentration of TRI in blood and brain observed during the 30-minute exposure period.

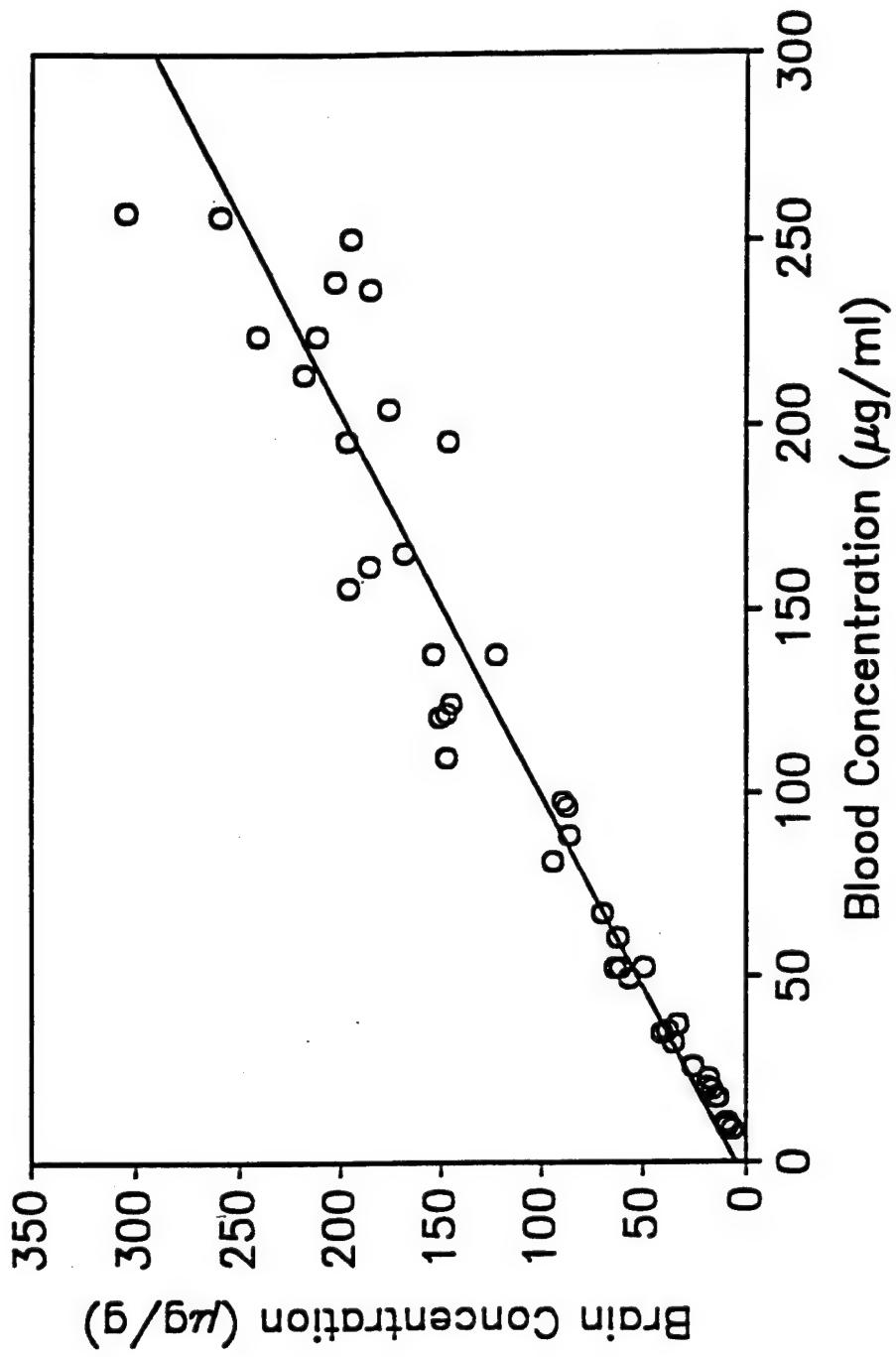


Figure 2: Scatter plot relating blood and brain concentrations of TRI. Each data point represents the mean blood and brain concentration of two (12,000 and 14,000 ppm) or four mice after 6, 12, 18, 24 or 30 minutes of exposure to one of nine TRI concentrations. The equation of the regression line is: $y = 0.954x + 5.095$.

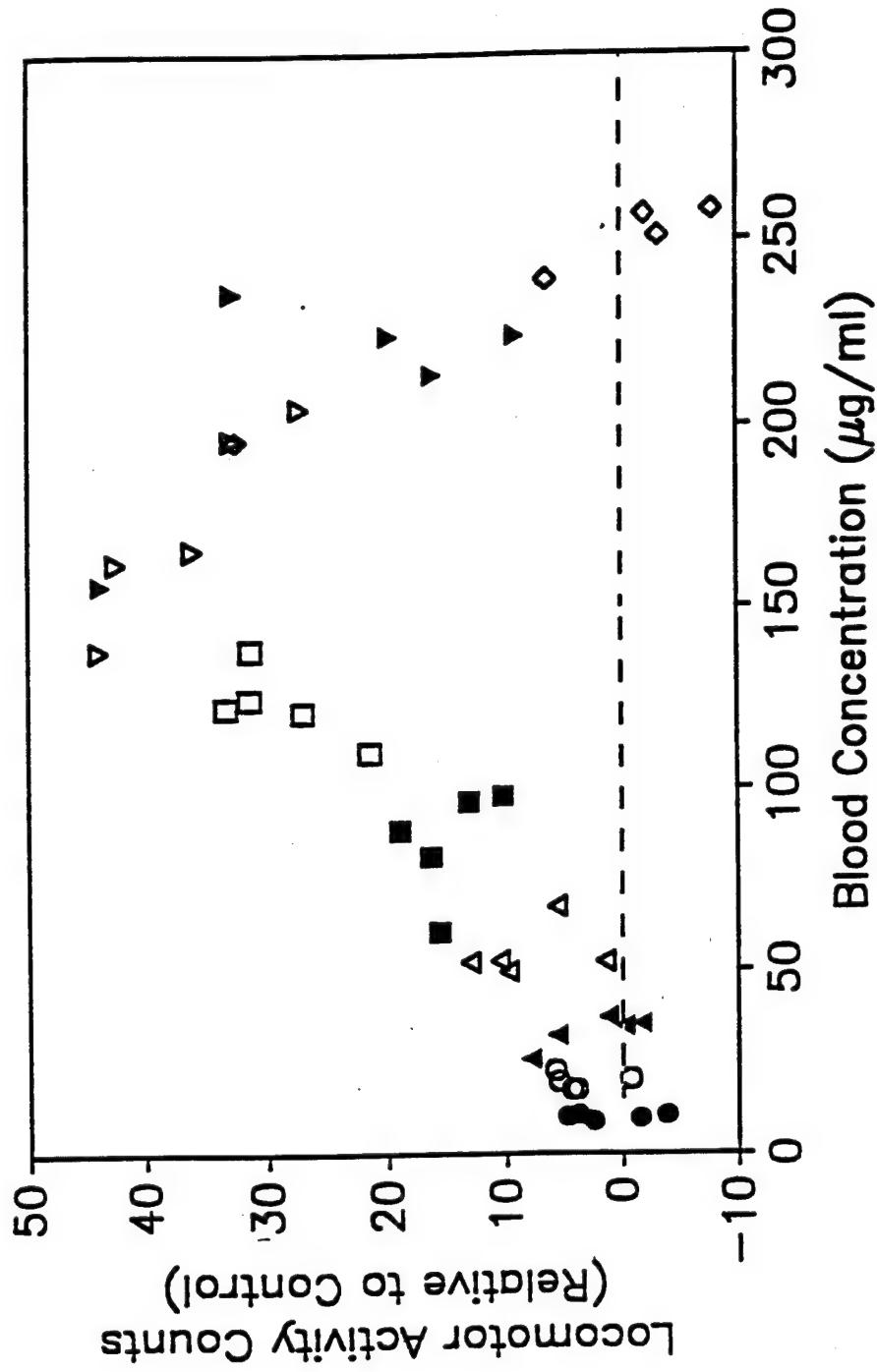


Figure 3: Scatter plot relating the blood concentration of TRI to locomotor activity. Each data point represents the mean blood concentration of two (12,000 and 14,000 ppm) or four mice after 6, 12, 18, 24 or 30 minutes of exposure to 500 (●), 1000 (○), 2000 (○), 4000 (▲), 6000 (■), 8000 (□), 10,000 (▽), 12,000 (▼), 14,000 ppm TRI (◇), as well as the mean locomotor activity of ten mice at corresponding times and exposure concentrations.

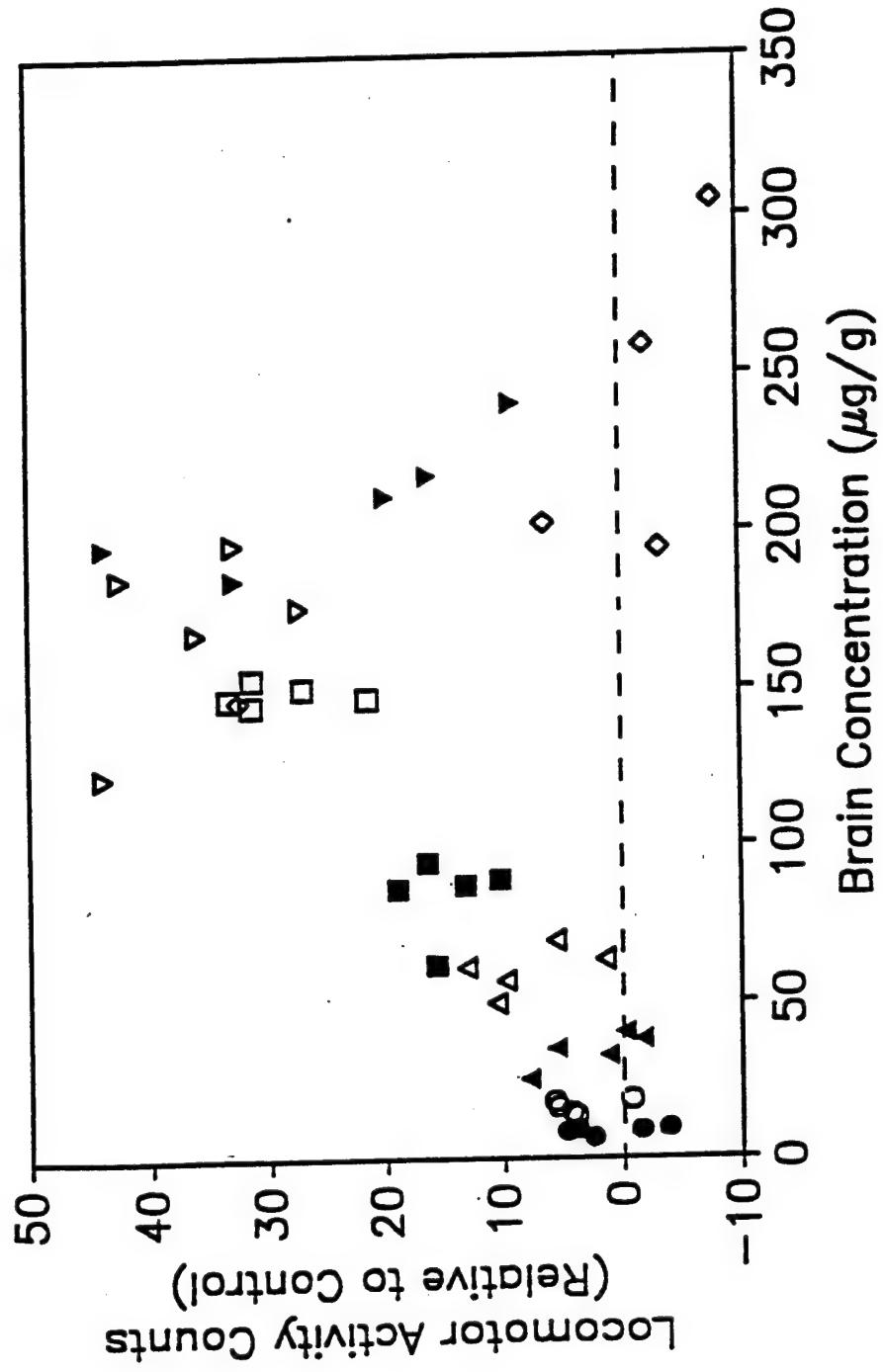


Figure 4: Scatter plot relating the brain concentration of TRI to locomotor activity. Each data point represents the mean brain concentration of two (12,000 and 14,000 ppm) or four mice after 6, 12, 18, 24 or 30 minutes of exposure to 500 (●), 1000 (○), 2000 (■), 4000 (▲), 6000 (△), 8000 (□), 10,000 (▽), 12,000 (▼), 14,000 ppm TRI (◇), as well as the mean locomotor activity of ten mice at corresponding times and exposure concentrations.

APPENDIX M

You, L., Muralidhara, S., and Dallas, C.E. "Regional brain distributions of trichloroethane in mouse and rats following inhalation exposures." To be submitted to the *Journal of Biochemical Toxicology*.

**Regional Brain Distributions of Trichloroethane
in Mouse and Rats Following Inhalation Exposures**

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ABSTRACT

Previous reports with toluene and ethanol indicate that volatile organic compounds (VOCs) have uneven distribution in various anatomically distinctive brain regions. No similar study has been reported for halogenated hydrocarbons, to which neurotoxicity is the most prominent health effect. It is known that substantial differences exist in TRI pharmacokinetics between species. In the present study, the uptake and distributions of 1,1,1-trichloroethane (TRI) in the brain tissue of mice and rats were examined. The animals were exposed to TRI at either 3500 or 5000 ppm for up to 2 hrs, and serial sacrificing were done at 10, 30, 60, and 120 min during each of exposure session. Seven brain regions from rats and 3 from mice were sampled, and TRI concentrations in the blood and brain tissues were determined by gas chromatography. Significant difference were found in the TRI concentrations between blood and brain tissue as well as among regional brain tissues in each species. Medulla oblongata in both species demonstrated overall the highest TRI concentrations, while cortex (in both species) and hippocampus (only sampled in rats) showed the lowest TRI concentrations. Substantial differences were also observed between the two species, as the mice exhibited higher capacity to accumulate TRI in the blood as well as in the brain tissues. The differential disposition of TRI among the brain regions is seen as mostly driven by the lipid content of various regions. Physiological difference in the respiratory systems of the two species and the physiochemical properties of the chemical favoring diffusion towards lipid-rich compartments might also have facilitated the species differences.

INTRODUCTION

1,1,1-Trichloroethane (TRI) is a volatile organic compound (VOC) that is widely used as solvent with many industrial and commercial applications. Because of their ubiquitous usage and potential for human inhalation exposure, VOCs as a class have long caused concern for their potential to incur detrimental health effects (Ikeda, 1992). As one

of the most frequently utilized VOCs however, TRI is considered one of the safer compounds compared to other halogenated hydrocarbons. At high dose levels, it has been demonstrated that TRI is capable of producing toxic effects in several organ systems. Cardiovascular toxicity induced by TRI exposure in laboratory animals has been shown by Reinhardt et al. (1973) and Herd et al. (1974); and hepatic and renal toxicities were reported by Plaa and Larson (1965), Klaassen and Plaa (1966, 1967), and Gehring (1968). More relevant to human exposures is that acute and high dose exposure to TRI in animals has been shown to cause disruptions on the functions of the central nervous system (CNS) following inhalation exposure (Arlien-Søborg, 1992a, Dobson and Jensen, 1992). Both neurobehavioral depressions (Evans and Balster, 1993; You et al., 1994a) and neurochemical alterations (Nilsson, 1986 & 1987; Kyrklund and Haglid, 1991) have been demonstrated following TRI exposures.

As a nonpolar, aliphatic, short chain molecule, TRI is highly lipophilic and volatile. Inhalation therefore is a major route of exposure in environmental and occupational settings, and systemic uptake of TRI upon inhalation is rapid and has the tendency to be more concentrated in highly lipid-rich organs or tissues (Dallas et al., 1989). About a 20 fold difference has been shown between total TRI uptake in fat tissue and that in most organ organs during a 100 min inhalation exposure session at 3500 ppm, and there were also differences, although with much smaller degree, among other types of tissues, such as between liver and muscle tissues (You et al., 1994b). This differential uptake and distribution of TRI among various organs can be largely attributed to factors like tissue lipid content and blood perfusion rates and may be a common feature for other organic solvents with similar physiochemical properties as well (Dallas et al., 1994; Pyykko et al., 1977). These same factors can be expected to affect chemical disposition within one organ, such as in the brain. Indeed, it has been reported that the distribution of VOCs such as ethanol (Sunahara et al., 1978) and toluene (Gospe and Calaban, 1988) in various

brain regions are not uniform in animals, or in human (Ameno et al., 1992) following uptake and distribution.

The usage of internal dose, rather than administered dose or inhaled concentration, has been emphasized in recent years (Dallas et al., 1994; Reitz et al., 1988) in evaluating the pharmacokinetic relationships of toxicants regarding to data extrapolations between dose levels, exposure routes and testing species. While useful for many purposes, blood concentration measurement is often inadequate to differentiate the pharmacokinetic behaviors of VOCs in respect to different organs and tissues relative to important toxicological endpoints (Dallas et al., 1994). Tissue concentrations, therefore, are seen as more relevant to toxicodynamic parameters that are specific to particular organs or tissues, and this was largely the basis for the usefulness of physiologically-based pharmacokinetic (PBPK) modeling for TRI and other VOCs (Reitz et al., 1988). This internal dose approach, however, is hard to define when it comes to solvent-induced CNS toxicity, in light of the differential dosimetry of TRI and possibly other VOCs among the various anatomical brain regions. To better characterize the CNS heterogeneity that may form the basis for selectivity in chemical neurotoxicity, an analysis of regional brain distribution would be a useful approach. In fact, a regionally-base PBPK model was recently developed to describe the CNS dosimetry for 2,4-dichlorophenoxyacetic acid in the developing and maternal rabbit brain (Kim et al., 1995).

For a prototype organic solvent neurotoxicant like TRI, there has been a substantial body of data describing its neurotoxicity and pharmacokinetics. It is known that a number of its neurochemical effects are specific to certain brain regions (Nilsson, 1986 & 1987). However, there has been no data available to date on its chemical disposition among the various brain regions. This study, therefore, was conducted to fill an important data gap between systemic uptake of TRI and the pharmacokinetics of regional brain distribution. To facilitate species comparisons of this pharmacokinetic property, two rodent species (mice and rats) were used in this study.

METHODS

Animals:

Adult male CD-1 mice (30-35 g) and adult male Sprague-Dawley (275-325 g) rats from Charles River Breeding Laboratories (Raleigh, NC) were used in the study. The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. The mice were housed in standard polypropylene mouse cages and the rats in stainless steel cages in a ventilated animal rack. The animals were acclimated to the animal facility for at least one week before the experiments. Tap water and commercial rodent chow were provided *ad libitum* in the duration. The inhalation exposures were always carried out between 900 to 1200 hr.

Chemicals:

1,1,1-Trichloroethane of 99% purity and isoctane of 99.98% purity were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). Methanol of 99.8% purity and chloroform of 99.9% purity were purchased from Fisher Scientific (Fair Lawn, NJ). The purity of the chemicals were verified by gas chromatographic analysis.

Inhalation Exposures:

Inhalation exposures were conducted in 1.0 M³ Rochester-type dynamic flow chambers. Test atmospheres of TRI were generated by vaporization of the compound under different combinations of air flow- and temperature-controlled schedules. A steady flow of nitrogen gas carried the vaporized TRI from a flask into the exposure chamber influent air stream. The entire TRI generation system was enclosed in a specially fabricated safety box, which was maintained under constant negative pressure during inhalation exposures, as was the inhalation chamber. Exhaust air from the vapor-generation box and the inhalation chamber were vented to HEPA and activated charcoal filters, so that all air with any chemical was scrubbed before release to the environment. The exposure chamber was operated at flow rates of 7 to 15 ft³ per minute (1/4 to 1/2 change of the chamber volume per min), and a negative pressure of 20 to 30 mm Hg was maintained at

all times during operation of the chamber. The animals were placed individually into sets of wire-mesh cages during the exposures. The concentration of TRI in the exposure chamber was monitored by an on-line Miran 1B2 infrared spectrophotometer (Foxboro Analytical, South Norwalk, CT) on the level of the animal cages. Clean air was pumped through the chamber following the termination of the inhalation exposure.

TRI Analysis:

The animal were exposed to either 3500 or 5000 ppm TRI for either 10, 20, 40, 60, 80, or 100 minutes. At each time point blood samples (0.5 ml for mice and 1.0 ml for rats) were withdrawn by closed chest cardiac puncture immediately after the animals were sacrificed by cervical dislocation. Intact brains were quickly removed and dissected on a glass plate, which was maintained on ice. Rat brains were dissected into seven discrete regions, while mouse brains were dissected into three regions. Briefly, the removed brains were divided quickly (<1 min for a mouse brain and ~2 min for a rat brain) into medulla oblongata (MO), cerebellum (CE), and cortex (CX) for mice and MO, CE, CX, hypothalamus (HY), striatum (ST), midbrain (MB), and hippocampus (HC) for rats according to Glowinsky and Iversen (1966). The dissected tissue pieces were immediately placed into ice-chilled scintillation vials containing 4 ml of isoctane and 1 ml of 0.9% NaCl and tightly capped. The collected samples were processed and analyzed in the same day of exposures. The tissues were homogenized using an Ultra-Turrax SDT homogenizer (Tekmar, Cincinnati, OH) for approximately 10 seconds. The vials were then vortex-mixed for 30 sec and centrifuged at 1800 x g for 10 min at 4 °C in capped vials. Aliquots of the isoctane supernatant layer were transferred into 20 ml headspace vials, which were capped immediately with teflon lined rubber septa and crimped to insure an airtight seal. The capped vials were then placed into a headspace autosampler unit of a Perkin Elmer Model 8500 gas chromatography (GC) (Perkin-Elmer, Norwalk, CT). Analyses were carried out using a stainless-steel column (182 cm x 0.317 cm) packed with 3% OV-17 (Alltech Associates, Deerfield, IL). The GC operating conditions were

headspace sampler temperature, 70 °C; injection port temperature, 150 °C; column temperature, 80 °C; and detector temperature, 360 °C. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 sec with the carrier gas, and a fixed volume of the isoctane supernatant injected automatically into the GC column. The limit of detection for TRI by the GC was less than 1 ng in 20 ml air, or 8.4 ppb TRI in the air of the sample vial. The recovery rate for TRI in blood and brain tissue from the sample processing procedure were previously determined (You et al., 1994b) using the method described by Chen et al. (1993).

Lipid Contents in Brain Tissues:

The method of Gospe and Calaban (1988) was used to determine the lipid content in each brain region. Separate groups of chemically naive rats ($n=5$) and mice ($n=6$) were decapitated, and their brain were removed and dissected in the same manners as described in the previous section. Sampled brain tissues were placed in the scintillation vials with a solvent mixture of Chloroform and Methanol (Chloroform : Methanol = 2:1). The amounts of solvent mixture used were in the proportion of 20 ml solvent per gram tissue. The tissue pieces were then homogenized for 10-12 seconds using the Ultra-Turrax SDT homogenizer, and the vials were then vortex-mixed for 30 seconds and centrifuged at 1800 x g for 10 min at 4 °C. After the supernatants were collected into pre-weighted glass tubes, the pellets were resuspended with the same amounts of solvent. They were centrifuged again, and the second supernatants were added to the first ones, which then were mounted on a N-Evap analytical evaporator (Organamation Associates, Northborough, MA) connected to a nitrogen gas tank. After the evaporation was complete, the glass tubes were weighed again. The difference between the second weights and the first ones were calculated as the lipid contents in the corresponding brain tissues.

Statistical analysis:

One-way analysis of variance (ANOVA), followed by a Fisher's least significant difference (LSD) procedure (with significance level set at $p<0.05$), was conducted to

detect the differences in TRI brain tissue concentrations in different brain regions and different exposure time points. An Independent t-test was used for detecting the differences between the TRI concentrations in the two species and two exposure dose levels.

RESULTS

TRI concentrations were adjusted and maintained at the target concentration in the inhalation chamber before each exposure session began. One exposure session was conducted for each species at one of the target concentrations for 120 minutes, and the animals were taken out of the exposure chamber at either 10, 30, 60, or 120 minutes. The actual TRI concentration during the 120 minutes exposure time was 3453 ± 73 ppm (Mean \pm SE) for the group of mice with a target concentration of 3500 ppm, 4966 ± 31 ppm for mice at 5000 ppm, $3509 \text{ ppm} \pm 52$ for rats at 3500 ppm, and 5026 ± 30 ppm for rats at 5000 ppm.

Uptake of TRI was very rapid, as TRI was detected in blood and every brain region at the earliest time point in both species. In general, the concentrations increased rapidly during the first ten minutes, which was the first measurement of the exposure, and gradually approached a near-steady state over the 60 to 120 minute period. The rate of uptake in some brain areas (such as in rat medulla oblongata in the 3500 ppm exposure group) showed a continuous upward trend of TRI concentration change from 60 min to 120 min of exposure. The concentration measured at 60 min were mostly over 90% of the concentrations measured at 120 min.

In a comparison of the rapid uptake phase between species, the blood TRI concentration in mice increased more rapidly than that in rats for the first ten minutes. At this point there was 58.9 and 66.4 ug/ml for the mice at 3500 and 5000 ppm, respectively, versus 43.5 and 49 ug/ml for rats at 3500 and 5000 ppm, respectively (Fig. 1A & B). However, there was no statistically significant difference across the four groups (2 species

× 2 doses) of blood TRI concentrations at the first 10 min of exposures. The differences in blood TRI concentrations between the two dose groups of mice were also not significant through the two hours of exposure. The mice of the 3500 ppm group did have higher blood concentrations than rats at the same exposure level at 60 and 120 minutes, and the 5000 ppm mouse group also had higher blood TRI concentrations than rats exposed to 5000 ppm at 60 and 120 minutes ($p<0.01$ for both time points, one-way ANOVA and LSD).

Both species had similar concentration-time profiles in the region of the medulla oblongata (Table 1). At the first time point at 10 min, 5000 ppm group of mice had higher TRI concentration than the groups of 3500 ppm in both mice and rats ($p<0.05$, one-way ANOVA). Otherwise, the other medulla oblongata concentrations across species or dose levels were not statistically significant. However, TRI concentrations in mouse cerebellum were significantly higher than rat cerebellum in at least one of the corresponding dose levels at 10, 30, and 120 minutes of exposure ($p<0.01$ in each case, one-way ANOVA). Cortex had relatively lower overall TRI concentration in the three brain regions measured in both rats and mice. In all of the four time points measured, rat cortex in the 5000 ppm group displayed significantly higher TRI concentrations ($p<0.01$ in each case, one-way ANOVA) than the other three groups, which were not distinguished from each other.

In a comparison between brain and blood concentrations, mice cortex had higher TRI concentrations than the blood, but the difference was not significant. Both medulla oblongata and cerebellum, however, did have significantly higher concentrations than both the blood and cortex ($p<0.05$ for all cases, one-way ANOVA) (Table 2). All of the rat brain regions had significantly higher TRI concentrations than the blood starting at the first measurement at 10 min and lasting throughout the entire exposure sessions (Table 1). Of the 7 brain regions measured in rats, hippocampus, cortex and striatum demonstrated the lowest overall TRI concentrations during the time course at both doses. Medulla oblongata and midbrain showed the highest TRI concentrations. Significant differences in

TRI concentrations were detected at every time point between rat blood and any of the brain regions as well as among the brain regions (Table 1).

A dose surrogate of TRI in the blood and brain regional tissues was represented by the area-under-the-concentration-time-curve (AUCs) for blood and each brain region. The AUCs of blood and other tissues in their respective species and dose levels were used to generate a AUC_{bl}/AUC_{tissue} ratio (Table 3.) This ratio (defined as the partition coefficient of TRI between blood and brain regional tissues) represented the total disposition of TRI over the exposure period in that region relative to the blood concentration. A smaller value indicated a higher accumulation rate of TRI in that region. There were substantial variations in the partition coefficient values, which ranged from 0.33 (medulla oblongata of the 5000 ppm group) to 0.58 (striatum of the 3500 ppm group) in rats and 0.54 (medulla oblongata of the 5000 ppm group) and 0.88 (cortex in the 3500 ppm group) in mice.

The measurements of lipid content (percentage of wet weight brain tissues) in the brain regions ranged from 9.0% (hypothalamus) and 17.4% (medulla oblongata) in rats and 12.8% (cortex) and 16.4% (medulla oblongata) in mice. Using the AUC values (combined values of the 3500 and 5000 ppm groups and pooled from both mice and rats) to correlate their respective lipid content measurements, a linear trend could be produced with a correlation coefficient of 0.73 (Fig. 2).

DISCUSSION

This study demonstrated that the widespread environmental contaminant TRI has a significant degree of differential disposition among various brain regions in rodent species following inhalation exposure. This is in agreement with what has been learned in the limited number of previous studies about CNS distribution of other VOCs such as toluene (Gospe and Calaban, 1988) and ethanol (Sunahara et al., 1978). The current study, however, seems to be the first report on the regional brain distribution of the

environmentally important class of short chain, aliphatic halogenated hydrocarbons. By comparing two commonly used laboratory rodent species, there were also significant interspecies differences with respect to the pharmacokinetic profile of TRI in different brain regions.

Systemic uptake of TRI into the blood and all brain regions in both species was characterized by very rapid absorption. Significant concentrations of the inhaled chemical were evident at the first time point of ten minutes, and a near steady state was reached in some cases after 60 minutes of exposure. This uptake pattern was in accordance with previously reported studies with TRI (Dallas et al., 1989; You et al., 1994a & b). The blood TRI concentrations at 10 minutes of exposure was 58%, 55%, 69%, and 69% of that for the 3500 ppm and 5000 ppm group of mice, 3500 ppm and 5000 ppm group of rats, respectively. It was apparent that mice had higher blood TRI concentrations than rats, and the difference steadily increased over time at both dose levels (Table 1 & 2). Mouse TRI concentration was 35.1% higher at 10 min and 60.2% higher at 120 min than their respective rat values in the 3500 ppm group, and in the 5000 ppm group, the comparisons at the same time points yielded 35.5% and 69.8% higher concentrations for mice. Although metabolic rates are greatly different between these two species, TRI is not metabolized to a significant extent and this would not be expected to be a major factor for these interspecies differences. The respiratory difference between the species is reflected in the alveolar ventilation, reported to be 5.11 liters/hr for rats and 1.26 liters/hr for mice in a previous study utilizing TRI (Reitz et al., 1988). On a body weight basis, this would be 280 ml/min•kg and 700 ml/min•kg for the rats and mice, respectively. Although increased uptake would be facilitated by a relatively higher alveolar ventilation, the elimination of TRI would also be increased, as respiration is the major route of elimination for TRI (Dallas et al., 1989). This factor is also reflected in the higher blood:air partition coefficient value for mice (10.8) relative to rats (5.75) (Reitz et al., 1988). This higher

blood TRI concentration in mice also seemed to result in higher partition coefficients in Table 3.

This study demonstrated that TRI has a differential distribution in specific brain regions upon systemic uptake, in a manner very similar to that of toluene (Gospe and Calaban, 1988; Ameno et al., 1992). The Gospe and Calaban study, one of the very few that have been published on regional brain disposition of VOCs, showed that in rat brain, toluene had its highest concentration in the medulla/pons area, followed by midbrain, cerebellum, thalamus, and frontal cortex. Hippocampus, caudate and hypothalamus areas were at the lower end of the concentration distribution spectrum. Ameno et al (1992) demonstrated that this toluene disposition pattern in brain regions was independent of route of administration (oral ingestion vs. inhalation), dose levels (inhalation of 2000 and 10000 ppm) and duration of exposure (0.5 - 10 hr). These investigators also examined human autopsy data that was associated with toluene poisoning and found remarkable similarities in the chemical's differential distribution in the brain regions of rats and humans. Our current study indicated that the ranking order of the partition coefficient values (Table 3) fits well with the regional toluene concentration ranking reported by Ameno et al. (1992). The values of brain regional/blood concentration ratio (BBCR), an indicator adopted in the Ameno report, seemed slightly higher with TRI than toluene when a compatible calculation method was used comparing the data in the two studies. For example, the BBCR were 2.7 and 2.0 for TRI in rat medulla oblongata and cortex, respectively, whereas they were 2.23 and 1.76 for toluene in the corresponding regions. This corresponds with the reported lipid:blood partition coefficient for TRI of 108 and 81-83 for toluene at 37°C (Arlien-Søborg 1992a & b).

Due to the limited amount of tissue mass in the mouse brain regions, TRI was measured in only three mouse brain regions. Medulla oblongata had the highest TRI concentration, followed by cerebellum and cortex. While this rank order apparently fits well with the TRI distribution pattern in rat brain, there were other differences in the

distribution pattern of TRI among the brain regions between rats and mice. In addition to the apparent difference in the blood concentrations of the two species, which confirmed some previous studies (You et al., 1994a; Warren et al., 1993; Schumann et al., 1982), it seemed that there were also species-specific regional brain differences. At the 5000 ppm dose level, the mice had statistically higher TRI concentrations in cerebellum but lower concentrations in medulla oblongata and cortex than rats. It has been estimated that fat tissue represents about 4% body weight of mice and 7% body weight of rats (Reitz et al., 1988). In light of the primary role of lipophilicity in halocarbon pharmacokinetics, this difference could have contributed to the higher blood TRI concentration in mice due to a smaller fat compartment as a "sink" for TRI.

As brain tissue exhibits considerable cellular, morphological and chemical heterogeneity, many factors could have contributed to the differential disposition of TRI in the different brain regions. An important characteristic of halocarbon as a class in their pharmacokinetics is their high degree of lipophilicity. This lipophilicity seems to be a predominant factor in determining regional uptake pattern of VOCs in the brain. Gospe and Calaban (1988) found that the toluene concentrations and lipid contents in the brain regions of Long-Evens rats were highly correlated and the R^2 value derived from them was over 0.9 (Measurements were taken at a single time point). In the current study using Sprague-Dawley rats and CD-1 mice, the lipid contents-TRI dosimetry (AUCs) correlation was not as high but still substantial ($R^2 = 0.73$). When using single time point concentration measurements instead of AUC values, the R^2 value increases up to 0.80; and when the two species are treated separately, the values become 0.89 and 0.99 for rats and mice, respectively. The lipid content in the tissue of a particular brain region was seen largely as the relative composition of white/gray matter (Ameno et al., 1992). The white matter, which contains a large portion of highly lipid-rich myelin in its neural tissue, appears more predominantly in areas such as medulla oblongata/pons where neural tracts are bundled together. The gray matter more predominantly occupies regions such as

cortex and thalamus/hypothalamus, and consists mainly of neuron cell bodies and unmyelinated neural fibers. Another factor that might have been expected to affect the regional brain distribution of TRI is the rate of blood perfusion in the different brain regions. It has been reported that while rat cerebellum and brainstem had similar blood flow rates, the perfusion for cortex was markedly higher than both (Gjedde et al., 1977; Sakurada et al., 1978). This is apparently in contradiction with the proportionality of TRI concentrations that we measured in those three rat brain regions, and therefore may indicate that the role of blood flow played in this regard may be limited. It was reported that differences in regional blood flow might have influenced the brain distribution of ethanol for the first 3 min after *i.p.* injection (Sunahara et al., 1978), but the high perfusion nature in the brain abated this effect very quickly thereafter. It suggests that for these type of compounds that are non-protein binding and with high solubilities in the brain, blood flow is not an apparent limiting factor in their uptake into the tissue.

Since CNS functions are highly organized on a structural and anatomical basis, it might be expected that regional distribution of TRI and other neuroactive halocarbons may have regionally related impact on physiological functions. Many of the neurotoxicological signs induced by toluene intoxication were disturbance of specific brainstem and cerebellar outflow, and that could be partially attributed to the early accumulation of toluene in the lipid-rich regions of medulla/pons and midbrain (Gospe and Calaban, 1988). Another aspect of the potential toxicological consequences of the differential disposition of VOCs is their possible interference with neurochemistry. Reported instances included regional specific effects of TRI on cyclic nucleotides metabolism (Nillson, 1986 & 1987). It was suggested that TRI might have effects on the functions of membrane-associated receptors due to altered membrane fluidity, and it seems feasible that these effects would depend on the amount of the chemical present. Yet another possible implication of the differential dispositions of VOCs in the brain is that it may affect the potential for biotransformations in brain tissues. Many VOCs such as

toluene and trichloroethylene are subject to extensive biotransformation in organs including the brain, and their metabolites have been implicated in their overall toxicity (Raucy, et al., 1993, Romanelli, et al., 1986). Further understanding of these interactions could contribute to the elucidation of the complex mechanisms of solvent neurotoxicity, and be useful in the health risk assessment neuroactive VOCs.

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TABLE 1. TRI Concentrations in Rat Blood (ug/ml) and Brain Regions (ug/g) ^a

	5000 ppm						
	10 Min ^b	30 Min ^b	60 Min ^b	120 Min ^b	30 Min ^b	60 Min ^b	120 Min ^b
3500 ppm							
Blood	43.6 ± 1.2	49.4 ± 6.2	55.9 ± 0.7	63.3 ± 1.4	49.0 ± 1.6	50.5 ± 2.8	67.9 ± 3.6
M. Oblong.	97.0 ± 5.1	131.4 ± 11.0	159.0 ± 8.9	182.5 ± 17.3	143.0 ± 20.5	157.6 ± 10.5	207.3 ± 30.0
Cerebellum	71.2 ± 4.5	85.9 ± 1.8	111.8 ± 4.9	112.6 ± 10.7	107.3 ± 4.2	108.0 ± 3.2	135.1 ± 7.1
Cortex	88.5 ± 9.0	100.6 ± 7.5	114.4 ± 5.1	119.7 ± 4.5	137.7 ± 10.4	140.7 ± 6.5	160.7 ± 15.7
Hypothal.	91.4 ± 4.1	109.6 ± 8.5	124.8 ± 5.8	131.1 ± 25.7	107.1 ± 6.8	127.3 ± 10.7	142.8 ± 5.0
Hippocam.	81.6 ± 12.8	102.5 ± 7.5	105.2 ± 6.9	105.6 ± 7.2	102.0 ± 13.4	119.2 ± 11.6	137.7 ± 10.9
Striatum	67.2 ± 6.7	78.0 ± 4.6	103.0 ± 7.8	110.7 ± 4.8	108.5 ± 6.8	132.9 ± 11.0	165.1 ± 6.3
Midbrain	103.2 ± 5.0	127.7 ± 13.2	139.9 ± 5.8	149.8 ± 3.1	132.3 ± 5.8	147.9 ± 8.3	175.6 ± 13.0

^a All values = Mean ± SE (n=5).^b Significant difference among TRI concentrations in rat blood and brain regions, p < 0.01, one-way ANOVA.

TABLE 2. TRI Concentrations in Mouse Blood (ug/ml) and Brain Regions (ug/g) ^a

	3500 ppm				5000 ppm			
	10 Min ^b	30 Min ^b	60 Min ^b	120 Min ^b	10Min ^b	30 Min ^b	60 Min ^b	120 Min ^c
Blood	58.9 ± 11.6	87.5 ± 12.9	92.2 ± 13.7	101.4 ± 19.9	66.4 ± 14.4	76.2 ± 11.5	98.8 ± 9.7	121.3 ± 11.0
M. Oblig.	96.3 ± 6.9	132.2 ± 12.6	173.0 ± 16.0	179.8 ± 6.8	121.4 ± 1.9	162.2 ± 17.4	185.1 ± 25.2	199.6 ± 27.9
Cerebellum	104.2 ± 5.1	141.8 ± 8.0	146.4 ± 14.8	151.1 ± 7.8	114.4 ± 5.3	151.0 ± 13.8	162.9 ± 20.7	192.6 ± 10.4
Cortex	67.0 ± 12.0	82.7 ± 7.2	102.1 ± 7.5	134.3 ± 4.7	91.6 ± 14.6	101.4 ± 11.4	102.7 ± 5.7	136.7 ± 14.8

^a All values = Mean ± SE (n=5).

^b Significant difference among TRI concentrations in mouse blood and brain regions, p < 0.01, one-way ANOVA.

^c Significant difference among TRI concentrations in mouse blood and brain regions, p < 0.05, one-way ANOVA.

TABLE 3. Partition Coefficient of TRI Between Blood and Brain Regional Tissues^a

bl = blood, mo = medulla oblongata, cb = cerebellum,

cx = cortex, hy = hypothalamus, hc = hippocampus,

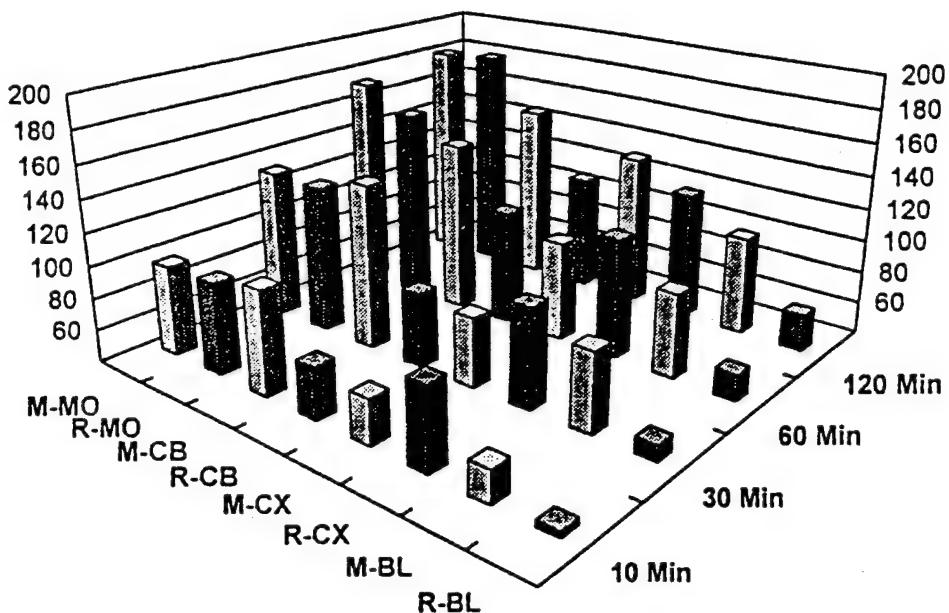
st = striatum, mb = midbrain

	Rat 3500	Rat 5000	Mouse 3500	Mouse 5000
AUC _{bl} /AUC _{mo} ^b	0.36	0.33	0.57	0.54
AUC _{bl} /AUC _{cb}	0.54	0.49	0.63	0.59
AUC _{bl} /AUC _{cx}	0.50	0.40	0.88	0.87
AUC _{bl} /AUC _{hy}	0.46	0.46		
AUC _{bl} /AUC _{hc}	0.54	0.48		
AUC _{bl} /AUC _{st}	0.58	0.40		
AUC _{bl} /AUC _{mb}	0.41	0.37		

^a The partition coefficient is expressed as the ratio of the area-under-blood-concentration-time-curve:the area-under-brain-region-concentration-time-curve.

^b Area under curves (AUC) were calculated from 0 to 120 minutes, assuming the TRI concentration at time 0 was 0.

(A) 3500 ppm Exposure



(B) 5000 ppm Exposure

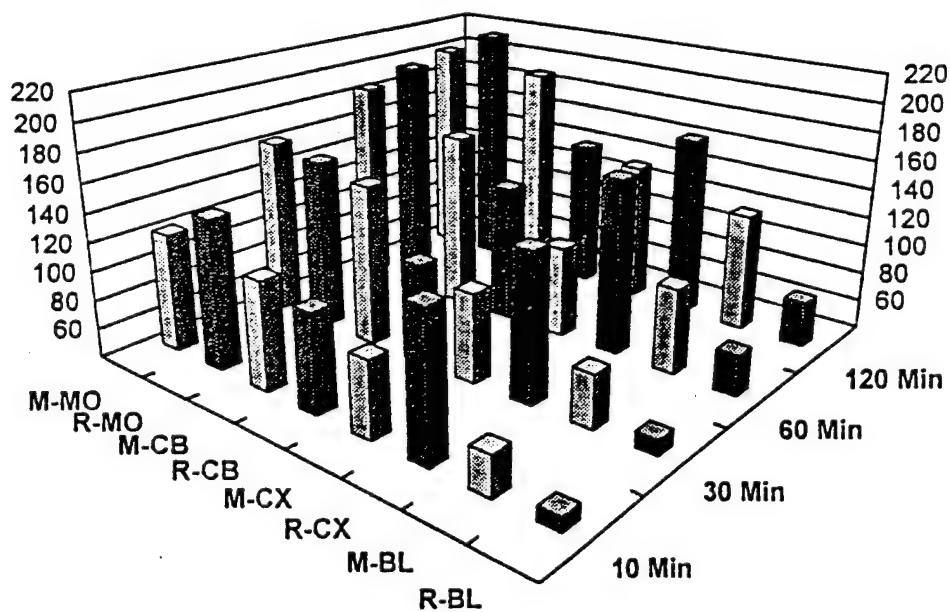


FIGURE 1. Blood and regional brain tissue TRI concentrations in mice and rats ($\mu\text{g}/\text{g}$ or $\mu\text{g}/\text{ml}$). M = Mice, R = Rats, MO = Medulla Oblongata, CB = Cerebellum, CX = Cortex, BL = Blood.

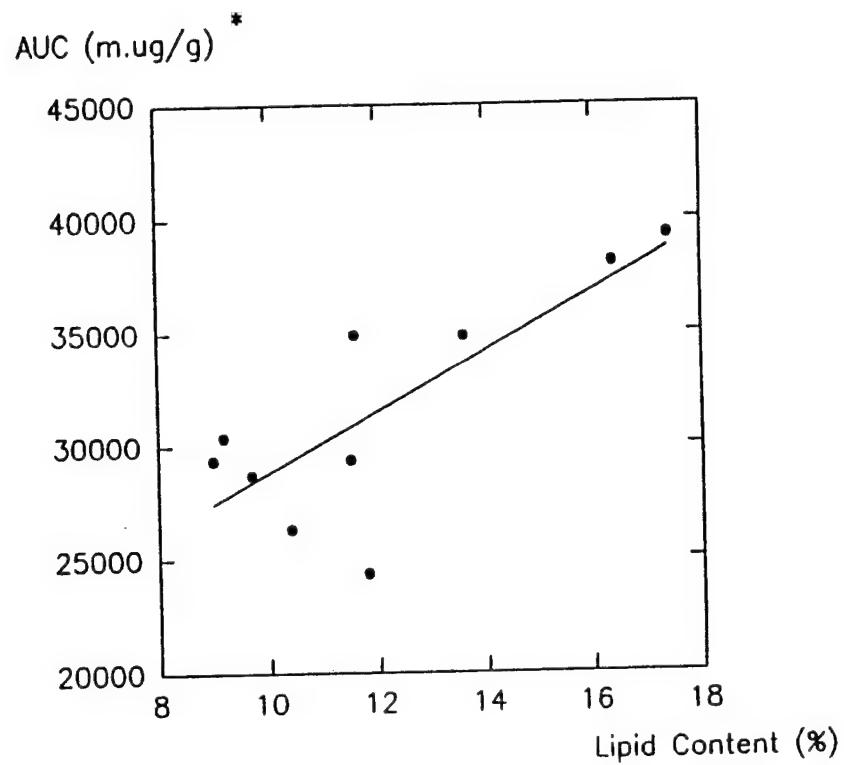


FIGURE 2. Correlation between brain regional lipid contents and TRI concentrations. The AUCs represent the sum of Area-Under-The-Concentration-Time-Curves for both 3500 and 5000 ppm groups. The data were pooled from mice and rats.

TABLE 1. Experimental Design: Inhalation Exposure Schedule and Concentrations.

Inhalation Dose Level				
	Target Concentration 2500 ppm		Target Concentration 4500 ppm	
	Actual Concentration		Actual Concentration	
Day	Kinetic ¹	GSTs ²	Kinetic ¹	GSTs ²
1	2495 ± 16	2463 ± 25	—	—
2	2486 ± 10	2490 ± 12	—	—
3	2512 ± 12	2515 ± 19	4576 ± 17	4489 ± 19

- 1 The rats were sacrificed at 6 time points during and following the exposure on the third day, and TCE concentrations in sampled tissues were measured;
 2 The rats were sacrificed 7 and 14 days after the last inhalation exposure; five unexposed animals served as control.

TABLE 2. TCE Concentrations in Regional Brain Tissues upon Inhalation Exposure

	30 MIN	60 MIN	120 MIN	POST 0.5 HR	POST 2 HR	POST 4 HR
2500 PPM						
MO	129.1 ± 5.8 ^c	173.8 ± 10.7 ^b	216.2 ± 10.0 ^e	134.3 ± 7.4 ^d	70.1 ± 8.1 ^b	19.9 ± 3.4 ^{b,c}
CE	97.0 ± 3.1 ^{a,b,c}	126.1 ± 6.8 [*]	156.7 ± 4.5 ^{b,c}	90.3 ± 3.1 ^{a,b}	47.7 ± 3.3 ^{a,b}	13.9 ± 2.1 ^{a,b}
HY	108.7 ± 2.9 ^{b,c}	198.0 ± 52.9 ^b	171.3 ± 8.4 ^{c,d}	103.1 ± 6.0 ^{b,c}	57.2 ± 6.3 ^{a,b}	22.2 ± 1.9 ^c
ST	109.5 ± 3.8 ^{b,c}	149.4 ± 6.3 ^{a,b}	183.2 ± 6.2 ^d	107.2 ± 6.1 ^{b,c}	59.6 ± 4.9 ^a	18.8 ± 2.0 ^{a,b,c}
MB	110.6 ± 4.7 ^{b,c}	117.4 ± 18.6 [*]	179.6 ± 7.2 ^d	122.7 ± 14.0 ^{c,d}	63.7 ± 6.7 ^b	18.3 ± 2.4 ^{a,b,c}
HC	111.6 ± 16.2 ^{b,c}	109.1 ± 6.9 [*]	143.2 ± 8.7 ^{a,b}	96.6 ± 5.1 ^{a,b}	49.8 ± 5.7 ^a	17.3 ± 1.6 ^{a,b,c}
CX	85.9 ± 3.1 [*]	105.6 ± 4.1 [*]	126.1 ± 6.1 [*]	75.8 ± 8.5 [*]	44.9 ± 3.9 [*]	12.7 ± 1.1 ^a
4500 PPM						
MO	229.2 ± 15.5 ^b	236.1 ± 22.8 ^b	337.7 ± 17.8 ^b	188.4 ± 10.3 ^d	89.5 ± 6.4 ^b	32.0 ± 2.7 ^b
CE	143.5 ± 21.4 [*]	138.1 ± 7.2 [*]	242.7 ± 16.2 [*]	135.5 ± 6.4 ^{b,c}	65.3 ± 4.2 ^a	24.6 ± 1.8 ^a
HY	225.0 ± 23.9 ^b	258.4 ± 32.9 ^b	360.2 ± 28.6 ^b	158.3 ± 4.9 ^c	71.7 ± 6.2 ^{a,b}	28.6 ± 1.3 ^b
ST	298.3 ± 38.7 ^c	317.3 ± 53.7 ^b	355.5 ± 55.0 ^b	149.8 ± 6.8 ^{b,c}	76.0 ± 6.0 ^{a,b}	28.2 ± 1.3 ^{a,b}
MB	106.2 ± 18.6 [*]	121.2 ± 29.9 [*]	190.5 ± 25.4 [*]	158.3 ± 9.5 ^c	80.0 ± 13.3 ^b	28.4 ± 3.1 ^{a,b}
HC	107.8 ± 16.7 [*]	143.6 ± 17.2 [*]	177.0 ± 33.1 [*]	126.4 ± 5.7 ^{a,b}	65.3 ± 5.4 ^a	26.1 ± 2.5 ^{a,b}
CX	119.2 ± 18.4 [*]	118.3 ± 24.4 [*]	157.7 ± 13.1 [*]	102.9 ± 12.9 [*]	63.2 ± 3.4 [*]	21.9 ± 1.5 [*]

Values are Mean ± SEM.

^{a,b,c,d,e} Values tagged by same letter have no statistically significant difference (One-way ANOVA and Duncan multiple comparison, p<0.05).

TABLE 3. TCE Concentrations in Blood and Extracerebral Organs upon Inhalation Exposure

	30 MIN	60 MIN	120 MIN	POST 0.5 HR	POST 2 HR	POST 4 HR
2500 PPM						
Lung	64.3 ± 5.1 ^a	87.8 ± 6.0 ^a	107.2 ± 6.0 ^a	88.2 ± 12.1 ^{a,b}	45.1 ± 3.9 ^b	13.5 ± 2.1 ^{a,b}
Liver	103.5 ± 9.2 ^b	137.5 ± 7.3 ^c	202.5 ± 6.6 ^c	121.2 ± 4.6 ^c	63.7 ± 6.3 ^c	17.8 ± 3.7 ^{b,c}
Kidney	107.2 ± 7.9 ^b	115.4 ± 4.1 ^b	156.2 ± 1.9 ^b	101.2 ± 2.3 ^{b,c}	66.1 ± 5.7 ^c	24.6 ± 3.7 ^c
Blood	58.1 ± 3.3 ^a	83.1 ± 2.8 ^a	106.2 ± 4.7 ^a	70.8 ± 3.2 ^a	25.4 ± 3.3 ^a	5.4 ± 1.0 ^a
4500 PPM						
Lung	260.5 ± 21.3 ^b	327.7 ± 97.8 ^c	448.2 ± 69.2 ^b	90.1 ± 17.3 ^a	50.9 ± 6.6 ^a	19.2 ± 1.8 ^a
Liver	132.2 ± 10.5 ^a	134.3 ± 17.8 ^a	230.6 ± 39.8 ^a	154.1 ± 27.1 ^b	84.5 ± 9.0 ^a	30.7 ± 2.4 ^{a,b}
Kidney	217.3 ± 43.1 ^b	212.9 ± 55.0 ^b	397.0 ± 37.0 ^b	125.3 ± 10.8 ^{a,b}	75.5 ± 18.8 ^a	30.9 ± 5.0 ^{a,b}
Blood	89.8 ± 3.9 ^a	106.5 ± 5.0 ^a	140.2 ± 15.5 ^a	91.6 ± 5.3 ^a	57.4 ± 7.2 ^a	22.8 ± 1.1 ^{a,b}

Values are Mean ± SEM.

^{a,b,c} Values tagged by same letter have no statistically significant difference (One-way ANOVA and Duncan multiple comparison, p<0.05).

TABLE 4. AUC Values of The TCE Concentration-Time Curves in Blood,
Brain Regions and Extracerebral Organs *

Brain Regions			Blood and Organs		
	2500 ppm	4500 ppm		2500 ppm	4500 ppm
CX	23.0	30.1	Blood	17.5	26.7
HC	26.8	33.7	Lung	21.5	54.6
CE	26.9	37.9	Kidney	29.9	51.3
MB	31.8	36.8	Liver	33.4	40.3
ST	32.1	57.9			
HY	33.4	53.3			
MO	38.0	55.3			

* AUCs were calculated using the trapezoidal rule. Values are min·mg/g tissue or ml of blood.

TABLE 5. Control Values of GST Subunits in Sprague-Dawley Rat Brain Regions

	SU 3&4	SU 7	SU 2	SU 6	SU 11	SU 8
MO	4.24 ± 0.08 ^a	18.54 ± 0.94 ^c	5.95 ± 0.24 ^b	4.14 ± 0.17 ^a	3.22 ± 0.10 ^{a,b}	2.87 ± 0.09 ^c
CE	12.36 ± 0.40 ^e	10.18 ± 0.39 ^a	3.19 ± 0.18 ^a	3.97 ± 0.14 ^a	5.06 ± 0.27 ^d	2.76 ± 0.14 ^c
HY	7.86 ± 1.05 ^d	18.61 ± 0.89 ^c	7.26 ± 0.81 ^c	8.47 ± 0.49 ^d	4.18 ± 0.38 ^c	3.83 ± 0.38 ^d
ST	5.81 ± 0.10 ^{b,c}	12.20 ± 0.72 ^a	4.72 ± 0.19 ^b	5.57 ± 0.26 ^b	2.80 ± 0.13 ^a	1.81 ± 0.14 ^a
MB	5.55 ± 0.25 ^{a,b}	14.80 ± 0.62 ^b	4.62 ± 0.32 ^b	4.08 ± 0.24 ^a	2.87 ± 0.18 ^a	2.41 ± 0.14 ^{b,c}
HC	7.29 ± 0.31 ^{c,d}	10.86 ± 0.37 ^a	7.85 ± 0.41 ^c	7.44 ± 0.30 ^c	3.10 ± 0.21 ^{a,b}	2.15 ± 0.11 ^{a,b}
CX	7.22 ± 0.50 ^{c,d}	12.15 ± 0.55 ^a	7.69 ± 0.51 ^c	6.65 ± 0.36 ^c	3.61 ± 0.19 ^{b,c}	2.11 ± 0.16 ^{a,b}
Standard Deviation	2.63	3.55	1.87	1.81	0.89	0.74

* Values are Mean ± SEM (N=5), expressed as pmol subunit protein/mg of cytosolic protein. ^{a,b,c,d}: Values tagged by same letter are not significantly different from one another.

TABLE 6. Control Values of GST Subunits in the Extracerebral Organs of Sprague-Dawley Rats

	SU 3&4	SU 7	SU 2	SU 6	SU 11	SU 8	SU 1	SU 1'
Lung	15.1 ± 1.5*	19.7 ± 2.5	18.0 ± 2.2	1.0 ± 0.1	0.8 ± 0.1	9.6 ± 0.5	ND	ND
Liver	171.6 ± 3.5	5.6 ± 0.6	99.8 ± 8.8	6.8 ± 0.6	ND	19.1 ± 1.9	128.0 ± 4.2	76.1 ± 10.6
Kidney	4.3 ± 0.2	53.4 ± 2.1	93.6 ± 5.2	1.3 ± 0.2	4.5 ± 0.1	27.1 ± 1.3	92.1 ± 3.7	8.1 ± 0.6

* Values are Mean ± SEM (N=5), expressed as pmol subunit protein/mg of cytosolic protein. ND = Not Detectable.

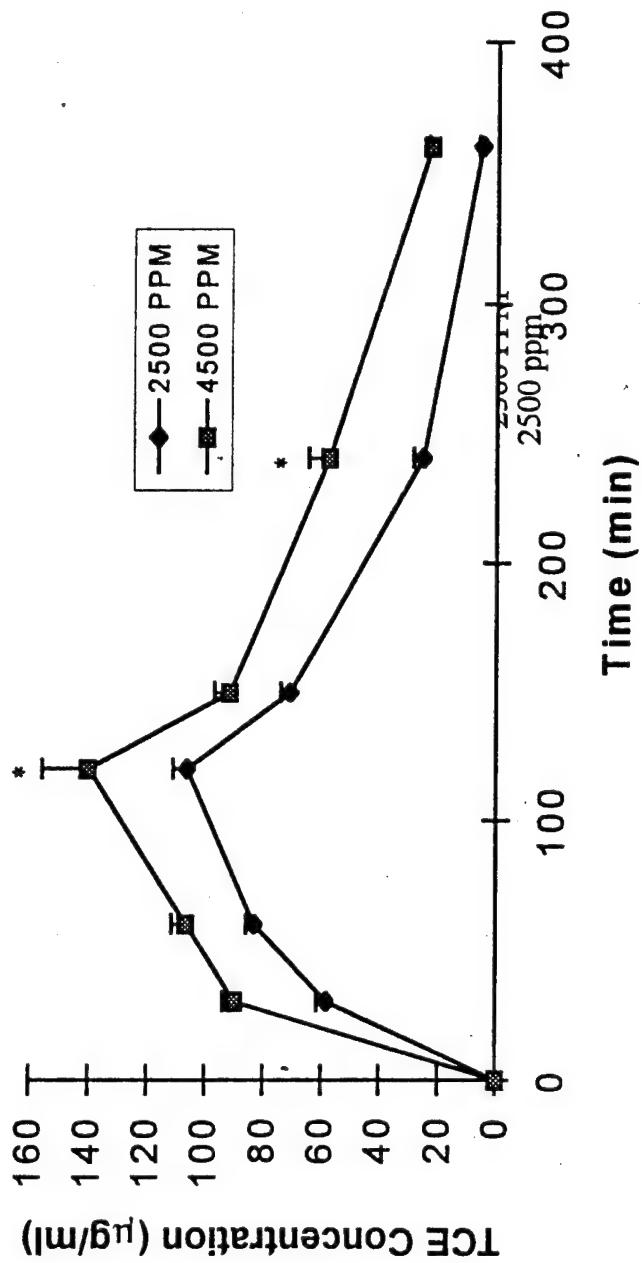


FIGURE 1. TCE Concentration-Time Profile in Rat Blood During and Following 120 Min Inhalation Exposure at 2500 and 4500 PPM. Values Are MEAN \pm SEM, (N=5). * indicate significant difference with the corresponding value at 2500 ppm ($p<0.055$)

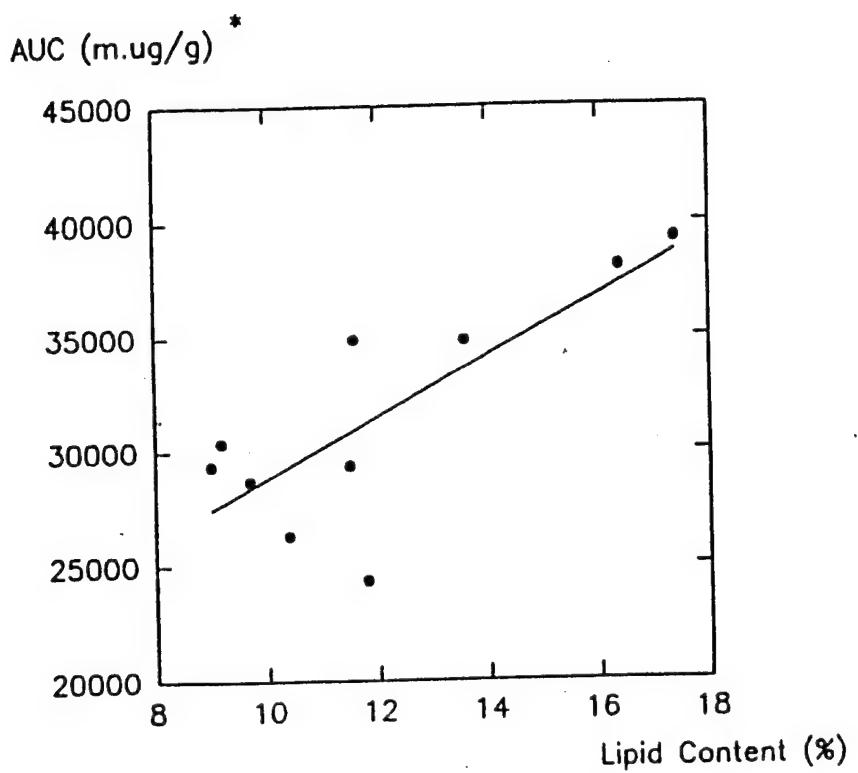


FIGURE 2. Correlation between brain regional lipid contents and TRI concentrations. The AUCs represent the sum of Area-Under-The-Concentration-Time-Curves for both 3500 and 5000 ppm groups. The data were pooled from mice and rats.

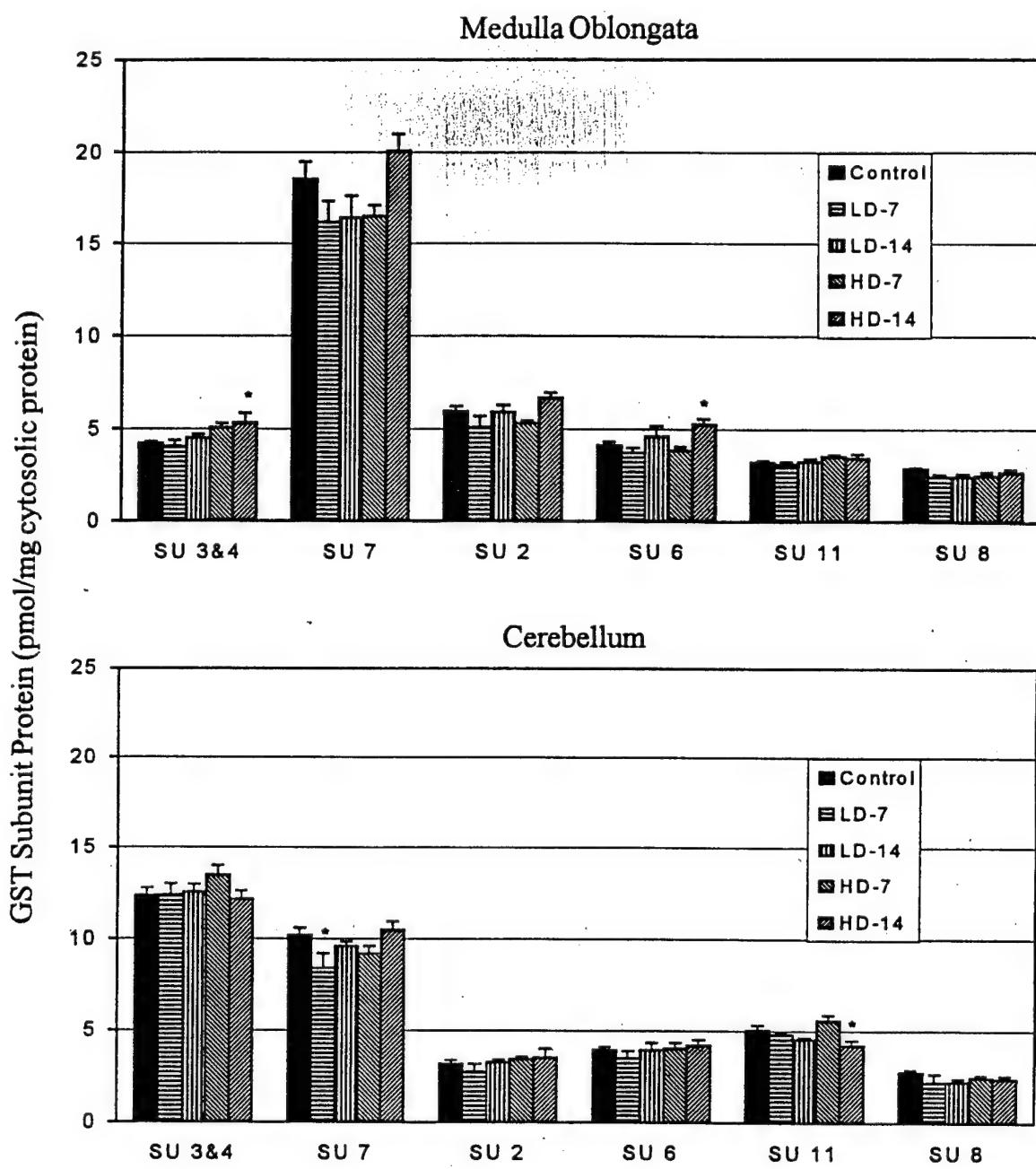


FIGURE 2. Effects of TCE inhalation on GST subunit protein concentrations in medulla oblongata and cerebellum. Rats were exposed to either 2500 ppm TCE, 120 min per day for 3 consecutive days or to 4500 ppm for a single 120 min exposure. Rats of each exposure level were sacrificed at either 7 or 14 days post the last exposure. The legend: LD (low dose) = 2500 ppm and HD (high dose) = 4500 ppm. Values are mean \pm SEM ($n=5$), and the * indicates significantly different from the control value (Duncan's multiple range test).

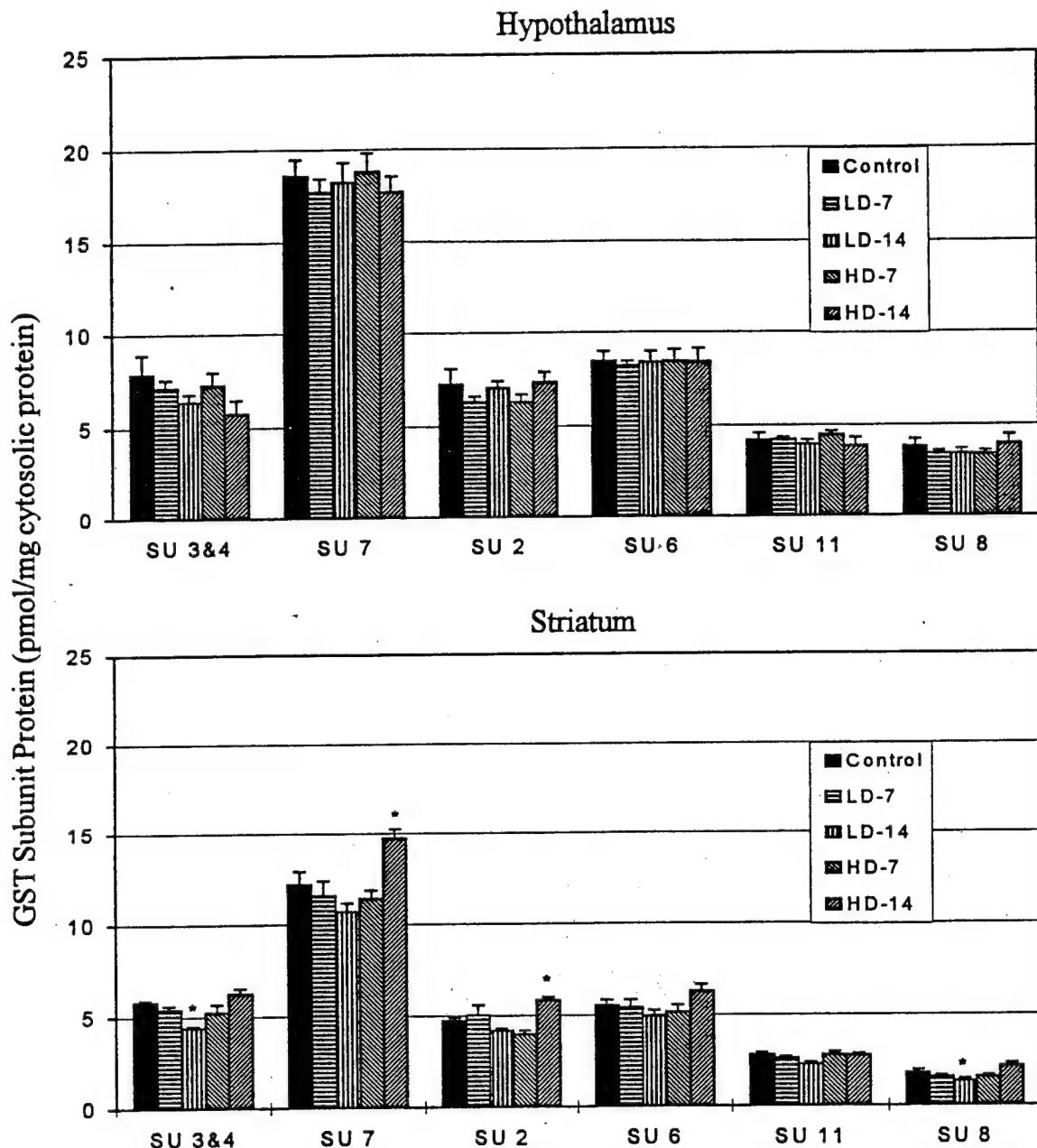


FIGURE 3. Effects of TCE inhalation on GST subunit protein concentrations in hypothalamus and striatum. Rats were exposed to either 2500 ppm TCE, 120 min per day for 3 consecutive days or to 4500 ppm for a single 120 min exposure. Rats of each exposure level were sacrificed at either 7 or 14 days post the last exposure. The legend: LD (low dose) = 2500 ppm and HD (high dose) = 4500 ppm. Values are mean \pm SEM ($n=5$), and the * indicates significantly different from the control value (Duncan's multiple range test).

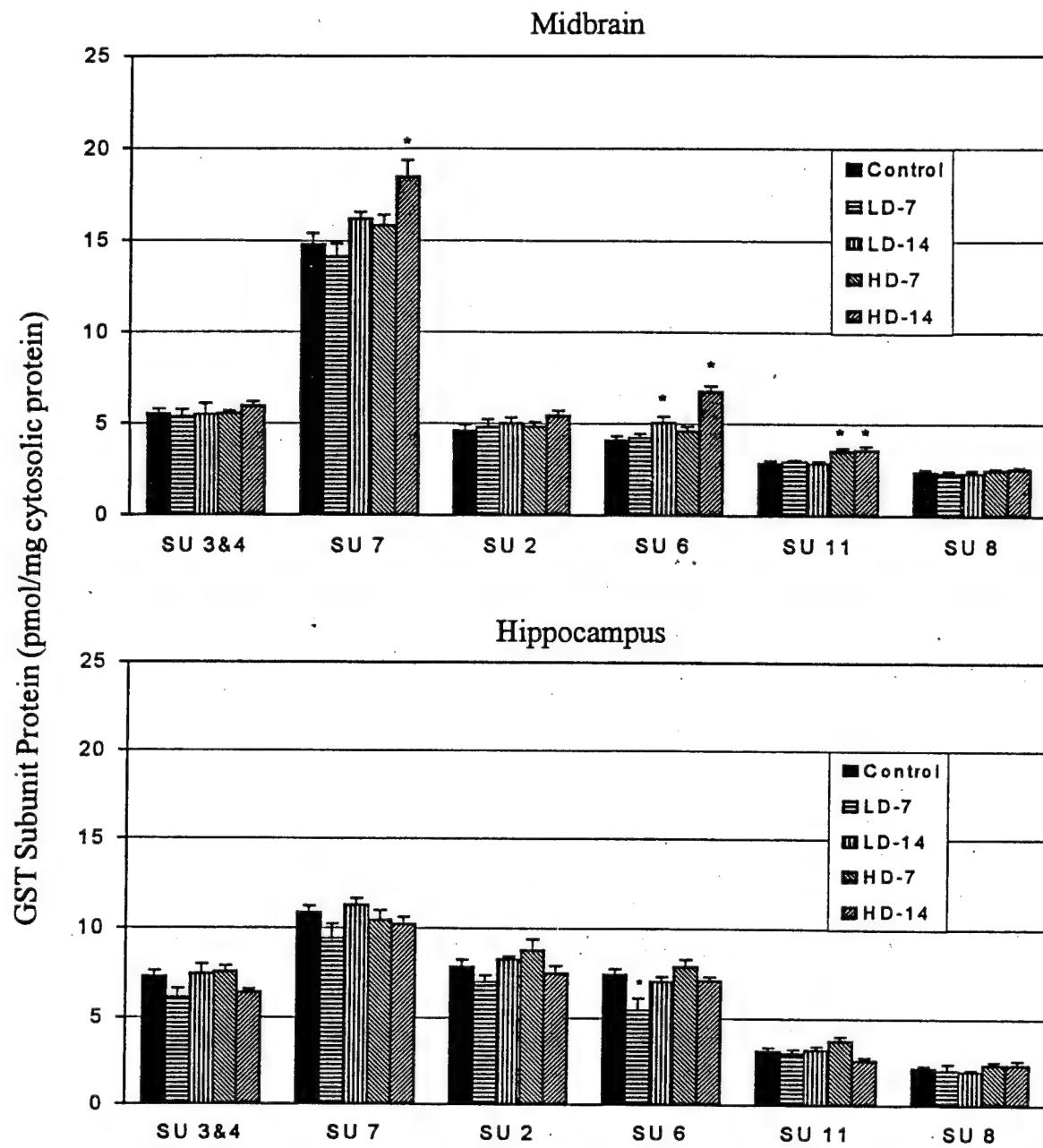


FIGURE 4. Effects of TCE inhalation on GST subunit protein concentrations in midbrain and hippocampus. Rats were exposed to either 2500 ppm TCE, 120 min per day for 3 consecutive days or to 4500 ppm for a single 120 min exposure. Rats of each exposure level were sacrificed at either 7 or 14 days post the last exposure. The legend: LD (low dose) = 2500 ppm and HD (high dose) = 4500 ppm. Values are mean \pm SEM ($n=5$), and the * indicates significantly different from the control value (Duncan's multiple range test).

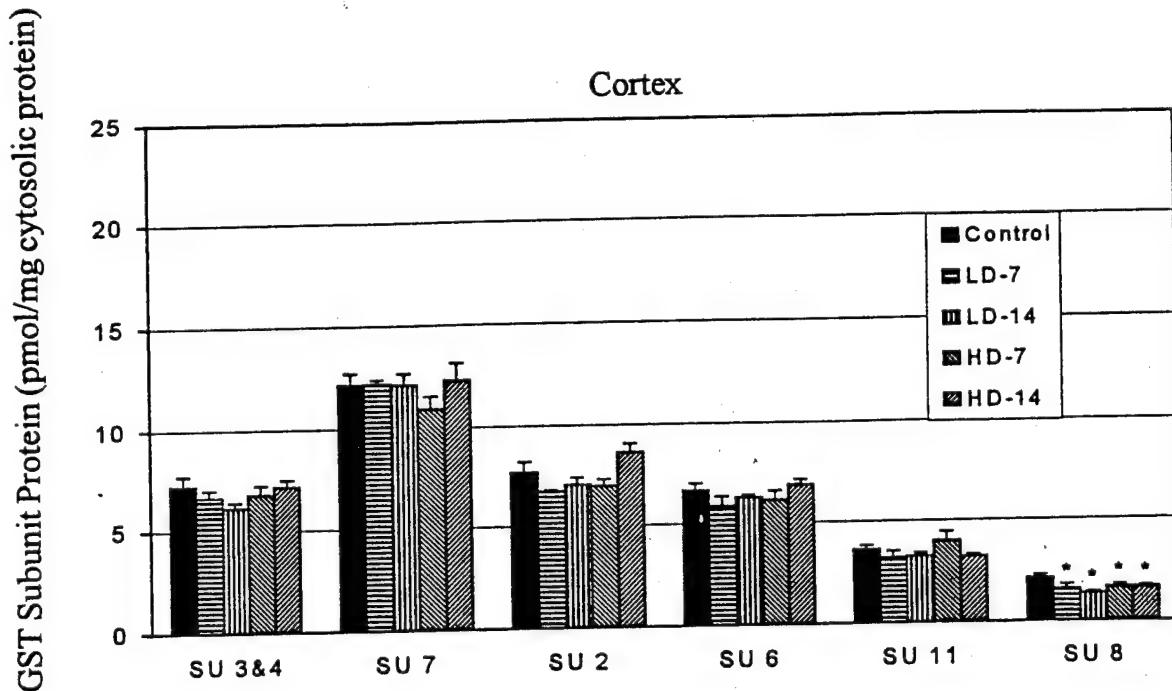


FIGURE 5. Effects of TCE inhalation on GST subunit protein concentrations in cortex. Rats were exposed to either 2500 ppm TCE, 120 min per day for 3 consecutive days or to 4500 ppm for a single 120 min exposure. Rats of each exposure level were sacrificed at either 7 or 14 days post the last exposure. The legend: LD (low dose) = 2500 ppm and HD (high dose) = 4500 ppm. Values are mean \pm SEM ($n=5$), and the * indicates significantly different from the control value (Duncan's multiple range test).

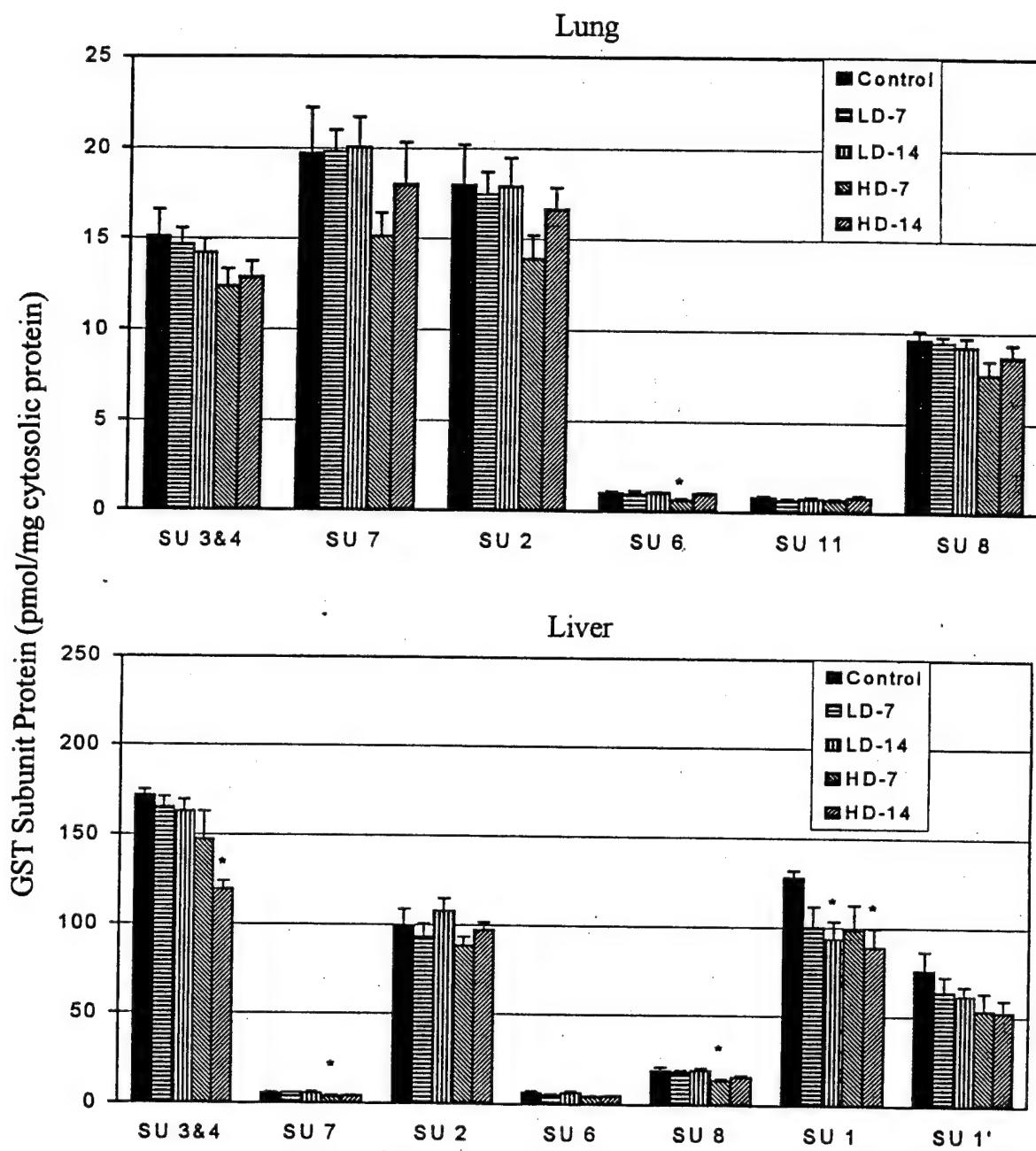


FIGURE 6. Effects of TCE inhalation on GST subunit protein concentrations in lung and liver. Rats were exposed to either 2500 ppm TCE, 120 min per day for 3 consecutive days or to 4500 ppm for a single 120 min exposure. Rats of each exposure level were sacrificed at either 7 or 14 days post the last exposure. The legend: LD (low dose) = 2500 ppm and HD (high dose) = 4500 ppm. Values are mean \pm SEM ($n=5$), and the * indicates significantly different from the control value (Duncan's multiple range test).

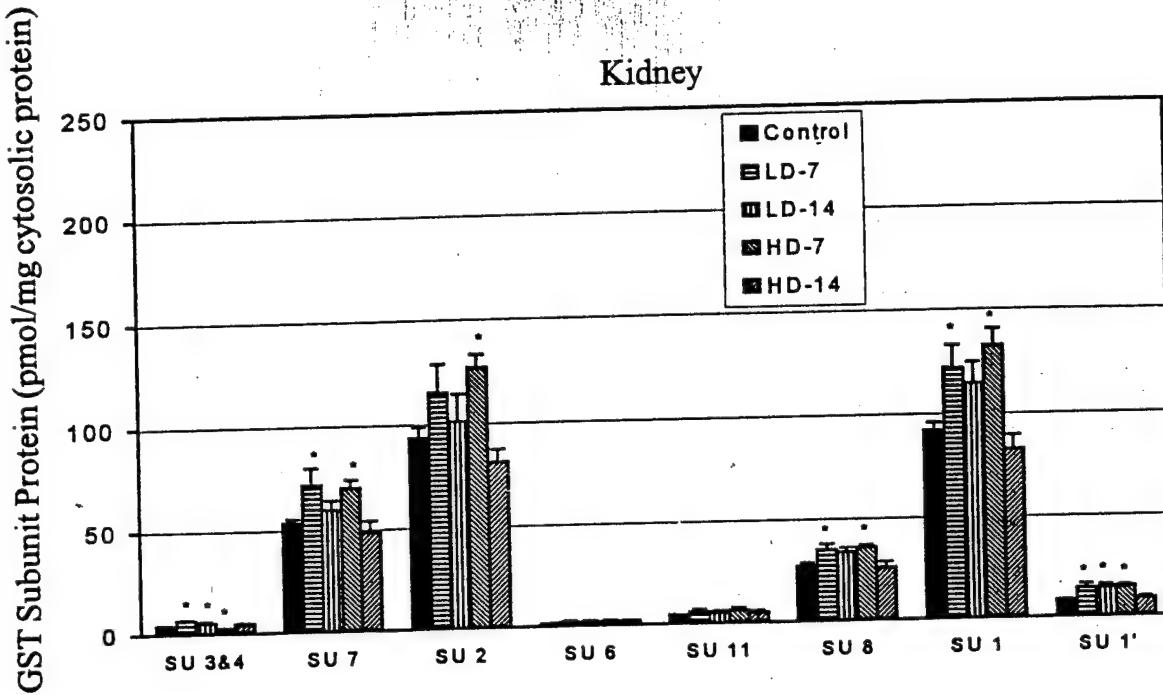


FIGURE 7. Effects of TCE inhalation on GST subunit protein concentrations in kidney. Rats were exposed to either 2500 ppm TCE, 120 min per day for 3 consecutive days or to 4500 ppm for a single 120 min exposure. Rats of each exposure level were sacrificed at either 7 or 14 days post the last exposure. The legend: LD (low dose) = 2500 ppm and HD (high dose) = 4500 ppm. Values are mean \pm SEM ($n=5$), and the * indicates significantly different from the control value (Duncan's multiple range test).

TABLE 7. Effects of TCE Inhalation on GST Activity in Brain Regions and Extracerebral Organs

	CONTROL	LD-7	LD-14	HD-7	HD-14
MO	0.213 ± 0.004 ^a	0.233 ± 0.007	0.230 ± 0.009	0.214 ± 0.005	0.248 ± 0.012*
CE	0.271 ± 0.009 ^{b,c}	0.278 ± 0.010	0.274 ± 0.016	0.299 ± 0.008	0.302 ± 0.007
HY	0.325 ± 0.019 ^d	0.328 ± 0.012	0.342 ± 0.014	0.351 ± 0.012	0.329 ± 0.011
ST	0.271 ± 0.020 ^{b,c}	0.261 ± 0.005	0.215 ± 0.022*	0.270 ± 0.014	0.274 ± 0.011
MB	0.251 ± 0.005 ^b	0.271 ± 0.003	0.286 ± 0.019*	0.296 ± 0.005*	0.297 ± 0.014*
HC	0.301 ± 0.005 ^{c,d}	0.306 ± 0.009	0.336 ± 0.015*	0.350 ± 0.008*	0.342 ± 0.007*
CX	0.246 ± 0.008 ^{a,b}	0.211 ± 0.013*	0.261 ± 0.007	0.255 ± 0.015	0.280 ± 0.009
	CONTROL	LD-7	LD-14	HD-7	HD-14
Lung	0.188 ± 0.007*	0.188 ± 0.013	0.174 ± 0.009	0.160 ± 0.006	0.164 ± 0.012
Liver	1.495 ± 0.042 ^c	1.492 ± 0.036	1.375 ± 0.065	1.374 ± 0.026	1.160 ± 0.021*
Kidney	0.423 ± 0.011 ^b	0.420 ± 0.017	0.434 ± 0.005	0.470 ± 0.002*	0.423 ± 0.024

GST activity was measured by assaying the conjugation of 1-chloro-2,4-dinitrobenzene with GSH and values are Mean ± SEM (N=5) expressed as nmol of 1-chloro-2,4-dinitrobenzene conjugated /min per mg of cytosolic protein. * indicates significant difference with the control value (Duncan's multiple range test, p<0.05); ^{a,b,c,d}: Values tagged with same letter are not significantly different from one another.

APPENDIX N

You, L., Muralidhara, S., and Dallas, C.E. "Effects of inhaled 1,1,1-trichloroethane on the regional brain cyclic GMP levels in mice and rats," to be submitted.

**Effects of Inhaled 1,1,1-Trichloroethane
on the Regional Brain Cyclic GMP Levels in Mice and Rats**

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ABSTRACT

As it is known that volatile organic compounds (VOCs) exhibit differential dispositions among anatomically discrete brain regions in rodents as well as in humans, potential toxicological consequences of this pharmacokinetic feature were evaluated using measurements of cyclic GMP. With the knowledge of 1,1,1-trichloroethane (TRI) uptake and distribution in the brain regions, cyclic GMP was evaluated due to its known susceptibility to the effects of organic solvents, its pivotal physiological role in perpetuating changes in neurochemical pathways and its possible involvement with neurobehavioral functions, whose disruption is one of the primary health effects associated with solvent exposures. Male CD-1 mice and Sprague-Dawley rats inhaled 5000 ppm TRI for 40 and 100 min in dynamic inhalation exposure chambers, and the brain procured from the animals immediately following terminating by microwave irradiation. Significant decreases in cyclic GMP levels were found in the cerebellum of both species, 55% and 58%, respectively, relative to the controls. There was a further decrease in both species after 100 min of TRI inhalation. Decreases of cyclic GMP with smaller magnitudes than in cerebellum were seen in the cortex of both species at both time points of measurement. A decrease of cyclic GMP was observed in the medulla oblongata of mice but not in rats after 40 min of exposure. Due to its signal transduction functions, it might be expected that the effects on cyclic GMP could directly impact neurological function. Comparison of the results of this study with the regional brain distribution of TRI and its effects on behavioral performance in previous studies by this laboratory appeared to indicate that alterations in brain cyclic GMP levels may be only involved with the neurobehavioral toxicity of TRI in an indirect fashion, and it appears to not be directly related to regionally differential dispositions of TRI in rodent brain.

INTRODUCTION

1,1,1-trichloroethane (TRI) is a widely used volatile organic compound (VOC) for many industrial and commercial purposes. In addition to their widespread presence in the environment and in occupational settings, a significant potential for inhalation exposures in worker populations, TRI and other VOCs also impose an additional health hazard as solvent abusers intentionally intoxicate themselves by inhaling the chemical vapors (Sharp, 1992; Balster, 1987). Regarded as relatively less toxic than most other VOCs, TRI has been shown, in very high doses, to cause adverse effects in liver, kidney (Lundberg et al., 1986; Gerace, 1981; Klaassen and Plaa, 1967; Klaassen and Plaa, 1966), and heart (Reinhardt et al., 1973). The primary target organ for TRI is considered to be the central nervous system (CNS). Profound behavioral changes have been demonstrated upon TRI administration in experimental animals (Moser and Balster, 1985; Mullin and Krivanek, 1982; Warren et al., 1996) as well as in humans (Stewart, 1968; Gamberale and Hultengren, 1973; Mackay et al., 1987) when TRI was administered at high dose levels. It is also known that a condition of chronic toxic encephalopathy, termed organic effective syndrome, could develop following long-term exposure to VOCs (Arlien-Søborg, et al., 1992).

It has been advocated that internal dose, i.e. the dose level that is actually delivered to the action sites in the target organ for a particular chemical agent, rather than administered doses be used in risk assessment (Dallas et al., 1994; Reitz et al., 1988). However, this concept of "internal dose", when the brain is the target organ, may come with a degree of ambiguity for some neuroactive compounds. It is possible that a neuroactive compound may act on multiple sites of the brain for a spectrum of pharmacological/toxicological effects; and this aspect of neurotoxicity can be compounded by the fact that many xenobiotics, including some VOCs, do not distribute uniformly in various brain regions. In fact, differential distributions among various brain regions have been documented for such chemicals as ethanol (Sunahara et al., 1978) and

toluene (Gospe and Calaban, 1988) after their systemic uptakes. It has also been demonstrated in our laboratory that TRI as well as trichloroethylene has a similar differential distribution pattern among rodent brain regions (You et al, 1995).

Cyclic GMP (cGMP) is an important intracellular signal transducer and is believed to be involved in mediating the effects of a number of neuroactive substances (Rao et al., 1993; Sethy and Oien, 1991; and Hoffman et al., 1989). Its broad spectrum of effectors and involvement with multiple receptor-mediated pathways make it susceptible to the effects exerted by many endogenous and exogenous factors (Dunwiddie and Hoffer, 1982). A dynamic balance of cGMP is pivotal for the maintaining of proper cellular functions, and disruption of this balance is therefore regarded as an expression of toxicity (WHO, 1986). A study by Nilsson (1986) showed that mouse brain tissue cyclic GMP levels were susceptible to the effect of TRI, as decreases in cGMP levels were observed in brain regions included the vermes, brain stem, and cortex in a time-dependent and dose-dependent fashion. However, this study only examined three brain regions, too few to evaluate the effects of TRI differential dosimetry in the brain.

There has been no apparent efforts to evaluate the potential toxicological consequences for the differential distribution of halogenated hydrocarbons across brain regions. This study was set to examine the effect, on a brain regional basis, of TRI exposure on the tissue levels of cGMP. Both rats and mice were used so that species comparison could be made regarding their susceptibility to TRI effects.

METHODS

Animals:

Male CD-1 mice (30-35 g) and male Sprague-Dawley rats (275-325 g) from Charles River Breeding Laboratories (Raleigh, NC) were used in the study. The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. The mice were housed in standard polypropylene mouse

cages and the rats in stainless steel cages in a ventilated animal rack. The animals were acclimated to the animal facility after their arrivals for at least one week before the experiments. Tap water and commercial rodent chow were provided *ad libitum* in the duration.

Inhalation Exposures:

Inhalation exposures were conducted in 1.0 M³ Rochester-type dynamic flow chambers. Rats and mice were exposed to 5000 ppm TRI for either 40 or 100 minutes. Test atmospheres of TRI (99% purity, J. T. Baker Chemical Co., Phillipsburg, NJ) were generated by vaporization of the compound under different combinations of air flow- and temperature-controlled schedules. A steady flow of nitrogen gas carried the vaporized TRI from a flask into the exposure chamber influent air stream. The entire TRI generation system was enclosed in a specially fabricated safety box, which was maintained under constant negative pressure during inhalation exposures in the same manner as the inhalation chamber. Exhaust air from the vapor-generation box and the inhalation chamber were vented to HEPA and charcoal filters, so that all air with any chemical was scrubbed before release to the environment. The exposure chamber was operated at flow rates of 7 to 15 ft³ per minute. The concentration of TRI in the exposure chamber was monitored by an on-line Miran 1B2 infrared spectrophotometer (Foxboro Analytical, South Norwalk, CT) on the level of the animal cages. Clean air was pumped through the chamber following the termination of the inhalation exposure. The control animals were placed into the inhalation chamber with only clean air flow for 40 min.

Brain Tissue Dissections:

At the end of each of the inhalation exposure periods, each animal was sacrificed immediately by applying focused microwave radiation generated by a Biostate microwave apparatus (Gerling Lab, Modesto, CA) on the head of the animals. The power level for the microwave radiation was 3.0 kw, 0.8 sec for mice and 1.5 sec for rats. The brains were then rapidly removed and placed on a ice-chilled glass plate. The mouse brains were

dissected into medulla oblongata (MO), cerebellum (CE), cortex (CX), and the rat brains into MO, CE, hypothalamus (HY), striatum (ST), midbrain (MB), hippocampus (HC), and CX according to the method of Glowinsky and Iversen (1966). The tissue mass taken was not more than 100 mg apiece. The dissected tissue pieces were then placed into 1 cm × 12 mm glass tubes and frozen immediately by placing the glass tubes into a dry ice and acetone mixture. These tubes were then sealed by parafilm and stored at -80 °C until use.

Cyclic GMP Assay:

All the tissue samples were assayed within two weeks of exposure for their cyclic GMP levels. Each sample was individually analyzed. The sample tissues were homogenized in 1 ml of 6% trichloroacetic acid with a glass homogenizer placed on ice. After centrifugation of the homogenates at 4 °C for 15 min, the supernatants were extracted with water-saturated ether. The samples were dried by lyophilization, and the residues were then dissolved with 200 ul 0.05 M sodium acetate buffer, pH 6.2. Duplicate assays using 100 ul each of the sample solution were carried out with a radioimmunoassay kit (NEN, DuPont, Boston, MA) and the radioactivities of samples were detected by a gamma counter (Gamma 5500, Bechman, Fullerton, CA). The results were derived using a standard curve that was generated by using 9 concentrations of cyclic GMP that ranged from 0 to 10.0 pmol/tube. The procedural recovery rate was determined by including trace amounts of [³H]-labeled cyclic GMP in the assays, and the final results were expressed in pmol/mg wet tissue.

Statistics:

One-way analysis of variance was conducted to detect the differences in cyclic GMP levels in different brain regions and different exposure time points. A Student-Newman-Keuls test was then carried out following a significant F test. An independent t-test was used for the TRI concentrations in different species and different exposure dose levels. The significant level for all the procedures was set at $p \leq 0.05$.

RESULTS

The final actual chemical concentration over the 100 min TRI inhalation exposure session was 5054 ± 69 (Mean \pm SEM). With the non-exposed control animals, it was found that cyclic GMP levels in different brain regions were not uniform. In both mice and rats, cerebellum was found to have higher cyclic GMP level than any of the other brain areas that were measured (Table 1). In mouse brain, the value of cyclic GMP in cerebellum was 0.32 pmol/mg, which was significantly higher than that of cortex (0.19 pmol/mg), but the difference of this value with the medulla oblongata measurement (0.24 pmol/mg) was not significant. In the control group of rats, the cyclic GMP level of 0.74 pmol/mg in the cerebellum was significantly higher than that of any other regions. The other tissues had relatively smaller differences among their cyclic GMP levels which ranged between 0.19 pmol/mg of striatum and 0.37 pmol/mg of cortex. The control level of rat cerebellum cyclic GMP was significantly higher than that of mice ($p = 0.008$). The outcome of statistical tests on the regional brain differences of cGMP levels in both species were summarized in Table 2.

The cGMP levels in all the mouse brain regions were depressed at 40 min following the initiation of TRI inhalation exposure (Fig. 1). The magnitudes of decrease in cGMP values as compared with their control regional brain values were 52%, 58%, and 13% for medulla oblongata, cerebellum, and cortex, respectively; and the decreases in the medulla oblongata and cerebellum were statistically significant. At 120 min following the initiation of exposure, the rate of change in the cGMP values in medulla oblongata and cerebellum diminished; as the medulla oblongata recovered a little (13%) from the 40 min value, and the value in cerebellum declined 14% from its 40 min value. There was a continuing reduction of cyclic GMP level in the mouse cortex from 40 to 120 min that resulted in a 42% decrease between these two measurements, but this difference was not statistically significant.

Changes in cyclic GMP concentrations in rat brain tissues are shown in Fig. 2. There was a large (55%) decrease of cyclic GMP in cerebellum relative to control values

after 40 min of exposure. Another statistically significant reduction (27%) at 40 min was found in cortex relative to control cortex cGMP levels. The decreases in medulla oblongata, hypothalamus, and midbrain at the 40 min time point were 13%, 10%, and 3%, respectively, of the controls. None of these changes were statistically significant. There were two regions which registered an increase in cyclic GMP level. From the lowest (0.19 pmol/mg) of the control values, the cGMP level of striatum increased 13% to over 0.21 pmol/mg after 40 min exposure. Cyclic GMP in hippocampus also increased from 0.26 pmol/mg in the control to 0.33 pmol/mg in the animals inhaling TRI for 40 min. Although this was a 22% increase, it was not statistically significant. In the 100 min exposure group, the upward change of cyclic GMP level in rat striatum continued to a resultant concentration of 0.28 pmol/mg, a 31% increase over the 40 min value and 48% over the control value, yet without statistical significance. A similar increase was also observed at 100 min in hypothalamus and midbrain, which reversed the slight decrease of the 40 min measurements. The increase of cyclic GMP level in hypothalamus to 0.31 pmol/mg was a statistically significant increase that was approximately 50% higher than the control value and 67% higher than the 40 min value. A further decrease of cyclic GMP concentration from the 40 min level were observed in cerebellum and cortex (for 25% and 35%, respectively) after 100 minutes of exposure. The outcome of statistical comparisons for the time effects of TRI exposure on the cyclic GMP levels in medulla oblongata, cerebellum, and cortex of both species are summarized in Table 3.

Interspecies comparisons showed that the rats had higher cyclic GMP levels in all three regions that were compared (medulla oblongata, cerebellum, and cortex), both in the control as well as each of the two exposure groups. While the difference cGMP levels in the regions of cortex and medulla oblongata were closer between the two species, large differences were found in the region of cerebellum. The values in rats were over two-fold higher than the corresponding values for mice, as the cyclic GMP levels in rat cerebellum were 131%, 154%, and 108% higher than mouse cerebellum for the control, 40 min, and

100 min groups, respectively. The resultant *p* values of the independent t-tests were 0.008, 0.023, and 0.036 respectively.

The measurements of extracerebral organ tissues also revealed decreases in their cyclic GMP levels (Table 4). The brain tissues were fixed by focus microwave radiation as the mean of euthanasia, and the likelihood of postmortem metabolism of cyclic GMP was therefore reduced to minimum. This was not the case for extracerebral organs, for there was no microwave fixation of enzymatic activities. The results of cyclic GMP measurements from lung, liver, and kidney were therefore expressed as fractions of their respective controls. The measured values showed no significant difference either for one organ over time or among the organs at one time point.

DISCUSSION

The present study indicated that tissue levels of cyclic GMP, which is an important cellular second messenger for membrane-mediated signal transduction events, is susceptible to the effect of TRI exposure. It has been shown that the effects of TRI exposure on brain tissue cGMP levels, and possibly other neurochemical parameters, is regionally specific. Furthermore, these regional based effects seem to be similar in cerebellum and cortex and dissimilar in medulla oblongata between the mouse and rats species examined. As an important mode of actions for xenobiotics to affect cellular functions, alterations in signal transductions involving cGMP have been associated with many toxic events. Cyclic GMP dependent pathways have been known to be involved in physiological functions such as vasodilatation (Jing et al., 1995) and neurotransmission (Frandsen et al., 1992). Unlike cyclic AMP, cGMP has more diverse effectors in the cell (Corbin et al., 1990), therefore it may be able to indicate a wider range of toxic effects. Although the precise cellular events mediated by cGMP in most cases have not been established, it is known that most treatments that alter the general functional state of the nervous system can affect the level of cGMP. This change of cGMP metabolism would be

expected to in turn have consequences in CNS function, due to its role in signal transduction (Dunwiddie and Hoffer, 1982).

While the mechanism for solvent-induced neurotoxicity is still largely unknown, it is believed that the primary toxicological action is a membrane-associated event (Evans and Balster, 1991; Arlien-Søborg et al., 1992). Close similarities have been shown between the CNS effects of organic solvents and that of classic CNS depressant drugs (Evans and Balster, 1991). Alteration in cellular signal transductions seems to be an important aspect in the effects of CNS depressants. Indeed, this has been demonstrated in the case of ethanol on its inhibitory effects on cellular calcium flux (Hoffman et al., 1989) and N-methyl-D-aspartate (NMDA)-activated cellular events (Lovinger et al., 1989). It is known that Cyclic GMP is a second messenger for NMDA (Frandsen et al., 1992; Wood and Rao, 1991) and the effect of ethanol on this excitatory amino acid have been demonstrated (Hoffman et al., 1989). Furthermore, a direct involvement of aliphatic organic solvents with cellular signal transduction has been demonstrated in a study in which TRI exposure was responsible for a change in cGMP levels of multiple brain regions (Nilsson, 1986). It was found that intraperitoneal injection of TRI (0.6-2.4 g/kg) in mice caused a dose- and time-dependent depression of cGMP. This effect was regional specific as the cerebellum (including vermis posterior and vermis anterior with hemispheres) was most severely affected. The decreases of cGMP in brain stem and cortex was significant but smaller in magnitude comparing to cerebellum (less than 1 fold change in these two regions versus up to 3 fold change in the cerebellum). The Nilsson (1986) study also used inhalation exposure of up to 27,300 mg/m³ TRI (5050 ppm equivalent). Significant decreases of cGMP levels were found in mouse cerebellum and cortex regions, but not in the brain stem. In the current study, a decrease of cGMP level was detected in mouse medulla oblongata upon TRI inhalation, but no effect was seen in the same brain region of rats. The Nilsson study (1986) also reported recovery of the cGMP brain tissue levels to their control values in all the brain regions affected. That

occurred 30 min to 1 hr after the termination of inhalation exposure. This fast recovery from the effects of TRI exposure indicates that the disruption of the dynamic balance of cGMP metabolism in brain tissue was temporary and irreversible, suggesting that chemicals like TRI may in part exert their effects by causing a shift in the mode of cellular signal transduction on a functional level.

The cellular level of cGMP has been associated with the functional state of neural pathways regulated by excitatory amino acid (EAA). It has been demonstrated that EAAs such as NMDA and glutamate are able to elevate cellular levels of cGMP in multiple neural cell types and are suspected to play an important role in mediating neurotoxicity induced by EAAs (Frandsen et al., 1992; Danysz et al., 1989). It has been shown that MK-801, a NMDA receptor antagonist, can disrupt operant behavior in monkeys (Paule, 1994). This suggests a possibility that a decreased cGMP level may be associated with MK-801 induced behavioral toxicity. This is in agreement with our experimental results, in which the behavioral toxicity of TRI is accompanied by a decreased cGMP level in the brain.

Another pathway of significant importance in the CNS that may have a component involving cGMP is the GABA receptor-mediated cellular events. It was demonstrated that activation of GABAergic neurons caused an inhibition effect on the cGMP level (Biggio et al, 1977). Classic CNS depressants such as the diazepams, which are known to act through a GABA receptor-mediated process, have been shown to cause a decrease of cGMP in rat cerebellum (Biggio et al., 1977). It has been argued that the neurobehavioral toxicity profiles of VOCs are very close to those induced by neurodepressant drugs (Evans and Balster, 1991). It seems that the similarities in pharmacological and behavioral effects produced by the CNS depressants and VOCs support the speculation that VOCs may have effects on the functions of GABAergic neural pathways (Evans and Balster, 1991). The demonstrated change in brain cyclic GMP levels in the current study and by others (Nilsson, 1986) seems to lend support to this possibility. While one inhalation dose was

used in this study, Nilsson (1986) indicated that the depression effects on the cGMP levels by TRI was dose- and time-dependent. However, the change in cyclic GMP levels and its possible association with GABA mediated effects may not be the primary mode of action responsible for the toxicological effects, but rather only reflect a larger disturbance on the function or integrity of a related event, such as membrane hemostasis.

As the mammalian brain has significant anatomical and functional heterogeneity, examining toxicological and pharmacokinetic parameters on a regional basis rather than in whole brain has obvious potential benefits. This has long been recognized and widely used for brain tissue neurochemical assays, and in recent years, this regionally based approach has also been adopted to characterize the pharmacokinetic profiles of toxicants in the brain (Kala and Jadhav, 1995; Ameno et al., 1992; Gospe and Calaban, 1988; You et al., 1995). Recently, a regionally-based physiologically-based pharmacokinetic (PBPK) model was successfully constructed for predicting 2,4-dichlorophenoxyacetic acid pharmacokinetics in rabbit brain (Kim et al., 1995). The PBPK approach represents a useful dosimetry tool that can accurately predict the pharmacokinetics of certain chemicals in different brain areas, taking into account exposure scenario differences in animal species, routes of administration, and dose levels. Neurotoxicological data with reference to regional brain differences could have high utility not only to understand the characteristics of a particular neurotoxicant, but also to provide relevance to and validation of corresponding predicting models for risk assessment of neuroactive agents in the workplace.

It was previously found in our laboratory that inhalation exposure of mice to 5000 ppm TRI caused a profound depression in their schedule-controlled operant behavior (You et al., 1994). This depression started at about 20 min into the exposure and became significant during 40 to 80 min into exposure. However, there was a recovery from the depression after 80 min, and this recovery was complete by 100 min into the exposure. This pattern of behavioral change in mice during the operant testing could not be solely explained by the concurrently measured TRI uptake into the mouse brain, as the TRI

concentrations steadily rose over the time period of inhalation exposure. While apparently related to the onset of behavioral change, the presence of TRI in the brain was not able to prevent the development of the tolerance observed during the exposure period. The current study, while showing drastic decreases of cyclic GMP levels in all the measured regions at 40 min, did not produce an upward rebound of cyclic GMP level at 100 min. This suggests that whatever relationship exists between the decrease of brain tissue cGMP levels and the disruption of operant behavior that did occur concurrently with brain uptake of TRI, it is apparently not the cGMP levels that determine the observed functional tolerance in operant behavior in mice.

In a companion pharmacokinetic study to the current investigation (You et al., 1995), it was found that there was an uneven distribution for TRI in rat as well as in mouse brain regions. The region of medulla oblongata had the overall highest accumulation of TRI over a 100 min inhalation exposure period. Uneven distributions of TRI were not only found among discrete brain regions within a species, but also between comparable brain regions in the two rodent species examined. The regional distribution pattern of TRI found in our study was in agreement with previous findings as reported by Gospe and Calaban (1988). The differences in the lipid contents of the brain regions was seen as the primary determinant for the differential distribution patterns of the highly lipophilic VOCs (Gospe and Calaban, 1988). However, the pattern of differential distribution of the chemical in brain regions bears no apparent correlation with the pattern of changes in cGMP levels in the corresponding brain regions. For example, cerebellum harbored more significant changes of cGMP levels in both rat and mice than medulla oblongata, but the concentration of TRI in the two regions showed a reversed relative relationship. This indicates that whatever insights that cGMP analysis may bring to the understanding of halocarbon neurotoxicity, cGMP level is apparently not a useful dose surrogate to assess chemical impact on rodent behavior or pharmacokinetics, at least in the parameters compared to in the companion studies done by this laboratory.

It is known that there were significant interspecies differences in operant performance between mice and rats during TRI inhalation exposure (You et al, 1994; Warren et al., 1993). This species difference was also shown in the uptake and distribution of TRI into different brain regions in these species (You et al., 1995). In respect to the cGMP analysis, the rats showed higher cGMP levels of control values in the regions that were measured in both species. The cGMP levels in cerebellum sustained the most reduction upon TRI exposure. This may partially be due to the high physiological levels of cGMP in this region (Dunwiddie and Hoffer, 1982; Palmer et al, 1981) and therefore higher susceptibility to the reduction upon challenge by the toxicant. It may also be associated with the fact that a major aspect of TRI neurotoxicity is the disturbance of motor activities. The patterns of change in cyclic GMP levels upon TRI exposure are very similar in the cortex of both mice and rats (Fig. 1 & 2). However, they are dissimilar for the region of medulla oblongata where a significant decrease was shown in mice along the exposure. The underlying mechanism for this is not known, but it indicates the different responsiveness of mice and rats to the effect of chemical exposure.

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Table 1. Cyclic GMP levels (Mean \pm SE, pmol/mg wet weight) in comparable brain regions in mouse and rats.

	Control		40 Min Exposure		120Min Exposure	
	Mouse	Rat	Mouse	Rat	Mouse	Rat
Medulla Oblongata	0.24 \pm 0.03	0.30 \pm 0.02	0.12 \pm 0.03	0.26 \pm 0.03	0.13 \pm 0.02	0.24 \pm 0.02
Cerebellum	0.32 \pm 0.05	0.74 \pm 0.09	0.13 \pm 0.03	0.33 \pm 0.06	0.12 \pm 0.01	0.25 \pm 0.03
Cortex	0.19 \pm 0.03	0.37 \pm 0.03	0.16 \pm 0.04	0.27 \pm 0.04	0.10 \pm 0.02	0.18 \pm 0.02

Table 2. Statistical Outcome for Cyclic GMP Levels upon TRI Inhalation Exposure: Regional Differences.

	Mice		Rats	
	F	p	F	p
Control	3.696	.056	11.904	.000
40 Min	.582	.574	2.364	.057
100 Min	1.137	.353	1.833	.129

Table 3. Statistical Outcome for Cyclic GMP Levels upon TRJ Inhalation Exposure: Effects over time.

	MO	CE	CX	HY	ST	MD	HC
	F	F	F	F	F	F	F
	p	p	p	p	p	p	p
Rats	1.228	.327	10.878	.002	9.582	0.003	.0487
Mice	6.53	.0121	14.11	.001	2.87	.0961	-

Table 4. Changes of cyclic GMP levels in lung, liver, and kidney tissues in mice and rats after inhalation exposure to 5000 ppm TRI (Values are ratios of exposed/control).

	Mice		Rats	
	40 Min	100 Min	40 Min	100 Min
Lung	0.67	0.78	0.74	0.71
Liver	1.0	0.95	0.96	0.92
Kidney	0.86	0.95	0.58	0.66

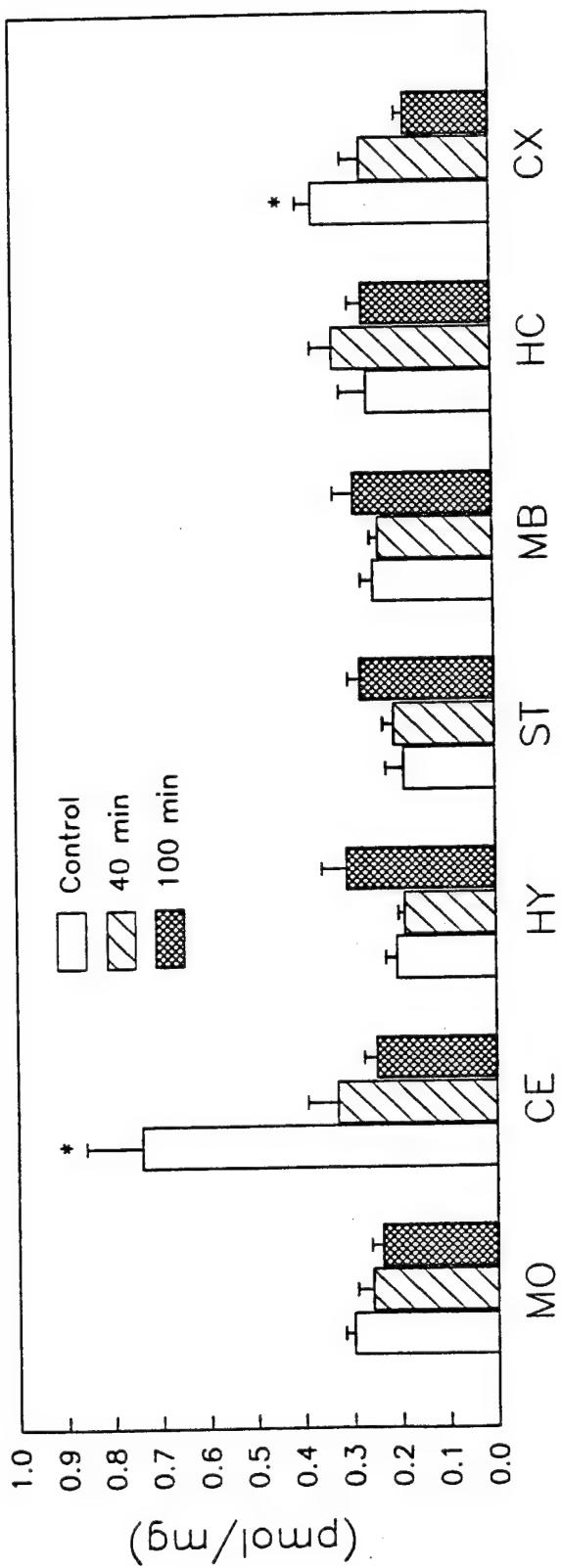


FIGURE 1. Cyclic GMP concentrations in rat brain tissues. MO=Medulla Oblongata, CE=Cerebellum, HY=Hypothalamus, ST=Striatum, MB=Midbrain, HC=Hippocampus, CX=Cortex. Values are Mean \pm SEM. * indicates significant difference with both 40 and 100 min values (ANOVA and Student-Newman-Keuls).

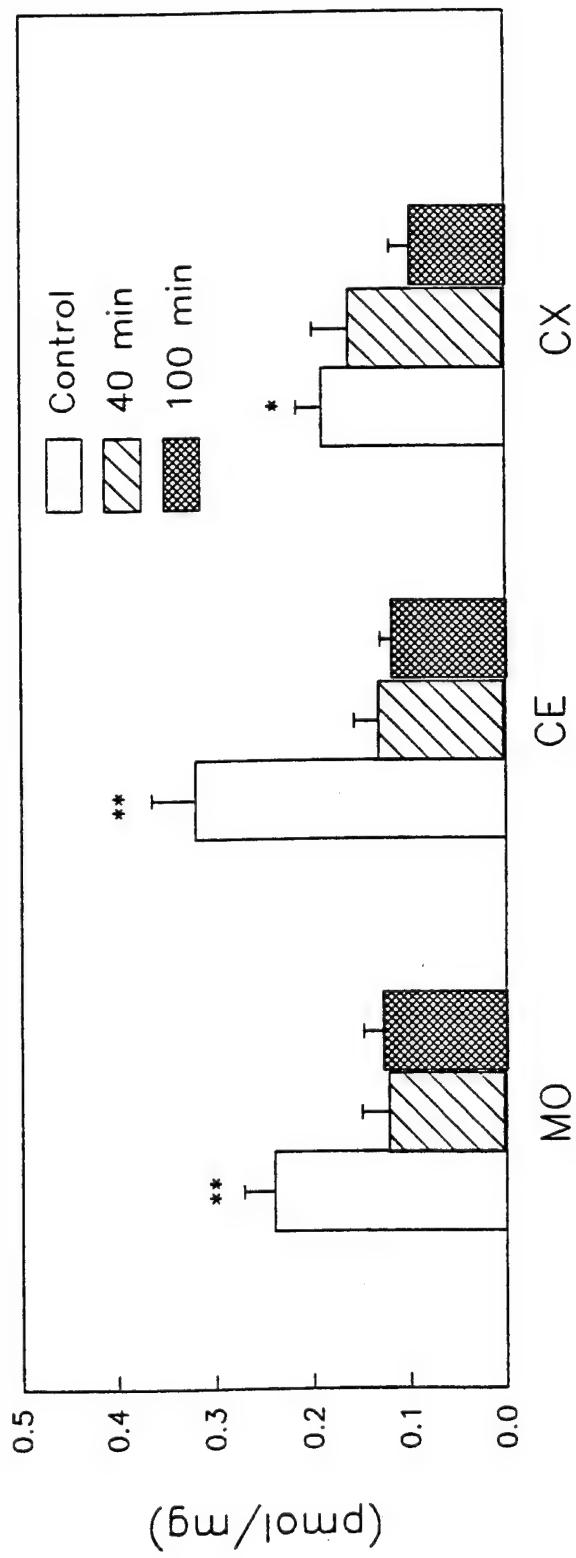


FIGURE 2. Cyclic GMP concentrations in mouse brain tissues. MO=Medulla Oblongata, CE=Cerebellum, CX=Cortex. Values are Mean \pm SEM. ** indicates significant difference with both 40 and 100 min values, and * with only 100 min value (ANOVA and Student-Newman-Keuls).

APPENDIX O

You, L., Muralidhara, S., and Dallas, C.E. "Rat regional brain dosimetry of trichloroethylene and its toxicodynamic relationship with glutathione S-transferase isozymes." To be submitted to *Fundamental and Applied Toxicology*.

**Rat Regional Brain Dosimetry of Trichloroethylene
and its Toxicodynamic Relationship
with Glutathione S-Transferase Isozymes**

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ABSTRACT

The tissue dosimetry of trichloroethylene (TCE) in Sprague-Dawley rat brain regions and extracerebral organs was examined and compared to the effects of TCE inhalation on glutathione S-transferase (GST) activity and its isoform protein levels in the brain. From the physicochemical properties of TCE and available data on the pharmacokinetic properties of similar compounds, it was hypothesized that TCE could exhibit differential regional brain concentrations in this target organ during the frame in which TCE-induced neurotoxicity has been demonstrated. Male Sprague-Dawley rats received either a single 2 hour inhalation exposure to 4500 ppm or three consecutive daily exposure to 2500 ppm for 2 hr each day. TCE uptake in brain was rapid following inhalation exposure, reaching levels more than twice that in the blood in the same time period. Regional brain differences in TCE concentrations were manifested, with medulla oblongata and striatum with relatively higher levels and cortex and cerebellum with lower concentrations. The effects of TCE inhalation on GSTs were not distinguishable between the repeated 2500 ppm exposure and the single 4500 pm exposure groups. While most changes in the GST subunit levels were increases in the later (14 days) time point of sampling, decreases of the protein levels were also observed. The relatively small magnitudes of these changes, in addition to the fact that there lacked an apparent correlation between the rank order of total TCE doses in the brain regions and the effects they brought about to the GSTs, indicates the possibility that the effects of TCE exposure on GSTs are nonsystematic and limited.

INTRODUCTION

Trichloroethylene (TCE) is a volatile halogenated hydrocarbon that has been used widely in the past as a solvent for a number of industrial and commercial purposes. Due to its prevalence in the environment, TCE has been extensively studied for its potential carcinogenicity and toxicological effects in various organ systems (Davidson, and Beliles,

1991; Brown et al., 1990). Like many other volatile organic solvents, TCE is known to have effects on the central nervous system (CNS) when high doses are administered to animal and humans (Tham et al., 1984; Richards and White, 1975; Phoon et al., 1984; Annau, 1981). The CNS effects of TCE are mostly depressant in nature, which accounts for its use in the past as a general anesthetic (Flanagan et al., 1990; Evans and Balster, 1991).

TCE is readily absorbed into systemic circulation upon inhalation or ingestion and is rapidly distributed throughout the body (D'Souza et al., 1985; Templin et al., 1995; Fisher et al., 1991). While the adipose tissue exhibits the highest uptake capacity of TCE (Eger and Larson, 1964), it is also known that the TCE levels in several organs exceeds that of blood (Savolainen et al., 1977). The difference between TCE concentrations in blood and organ tissues suggests that direct measurement of TCE at the target organ is more useful to establish needed dosimetry in relation to its target organ toxicity. It has been demonstrated with several volatile organic compounds that their uptake and distribution among various brain regions are uneven (Kim et al., 1995; Gospe and Calaban, 1988; Ameno et al., 1992; Sunahara et al., 1978). Few studies have contributed to an understanding of how this differential distribution of halogenated hydrocarbon in brain may contribute to the toxicological outcomes of inhalation exposure to those agents. Also, there has been no detailed evaluation of uptake and distribution of TCE among the discrete brain regions upon systemic absorption.

Glutathione S-transferases (GSTs) are multifunctional enzymes that are present in most mammalian organ tissues (Mannervik and Danielson, 1988). They have extensive physiological roles as intracellular binding and transporting proteins and in modulating the biological activities of various endogenous substances including prostaglandins, leukotrienes and steroids (Abramovitz et al., 1988; Listowsky et al., 1988). Their detoxification roles involve the metabolism of xenobiotics by catalyzing the second phase conjugating reactions between glutathione and a variety of compounds that possess an

electrophilic center (Parkinson, 1995; Philbert et al., 1995; Boyer, 1989). A number of cytosolic GST isoforms in various tissues and different species have been found and are grouped into three classes (α , μ , and π), each of which is believed to be the product of a separate genetic family (Boyer, 1989). Each of the cytosolic GSTs is a protein consisting of two subunits that are either identical or from the same class (Mannervik and Danielson, 1988). Their relative distribution or profiles are species- and tissue-specific and can be altered by a variety of biological and environmental factors such as neoplastic changes in the tissue or exposure to drug and environmental chemicals (Boyer, 1989).

It has been demonstrated that all the major classes of GSTs exist in the brain, and there are regional differences in the distribution patterns of α -, μ -, and π -class GST subunit expression (Johnson et al., 1993a; Abramovitz, et al., 1988). The presence of GSTs in the brain are regulated by both endogenous and exogenous factors, and they are regarded as capable of adaptive change in selective isozymes when confronted with toxicological insults such as in the case of hyperbilirubinaemia (Johnson et al, 1993b). The distribution of GSTs in the brain, with their toxicant conjugating ability, is therefore considered an important basis for differential susceptibility to neurotoxicants (Philbert et al., 1995). As most information on the regulation of GSTs comes from studies of the liver, in which a variety of chemicals are shown to induce the enzymes (Pickett and Lu, 1988), there is little information on the effects of xenobiotics on GST expression in the brain. The limited number of studies concerning neurotoxicant effects on GST activities did not report data on specific brain region or individual GST isoforms with which those effects were most closely related (Das et al., 1981). Since the brain is highly heterogeneous, regional GSTs data are deemed as critical in understanding chemical neurotoxicity (Philbert et al., 1995).

A major conjugation pathway for TCE metabolites is trichloroethanol glucuronide, which was reported to account for about 78% of total radioactivity in excreted bile, which was about 10% of the total dosage, in the first 24-hours after an oral dosing in rodents

(Green and Prout, 1985). There is also TCE-glutathione conjugate, a metabolite that was shown in animal studies as the precursor of N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine, a nephrotoxic compound; however, this TCE-GSH conjugation is a minor pathway, representing only about 1% of total TCE metabolites, and its formation in humans has not yet been confirmed (Bruckner et al., 1989; Dekant et al., 1990; Goepfert et al., 1995). Nonetheless, the relative minor quantitative contribution in TCE metabolism does not mean that GST expression may not be affected by TCE; in fact, it has been shown that lead, which is not a GST substrate, is capable of inducing GSTs in rat kidney (Moser et al., 1995).

With the consideration of regional brain kinetics of volatile organic compound and potential TCE effects on GSTs in the brain, this study investigated 1) the regional brain kinetics of TCE upon inhalation exposure; 2) the brain distributions of various GST isozymes; and 3) the potential TCE exposure to affect the expressions of those isozymes.

METHODS

Animals:

Male Sprague-Dawley (200-250 g) rats from Charles River Breeding Laboratories (Raleigh, NC) were used in the study. The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr, in stainless steel cages of a ventilated animal rack. The animals were acclimated to the animal facility for at least one week before the chemical exposures. Animals were randomly assigned to experimental groups measuring TCE pharmacokinetics and GST effects. Tap water and commercial rodent chow were provided *ad libitum* in the duration. The inhalation exposures were carried out between 900 to 1200 each time.

Chemicals:

Trichloroethylene of 99% purity and isoctane of 99.98% purity were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). The purity of the chemical were verified by gas chromatography.

TCE Vapor Generation:

Inhalation exposures were conducted in 1.0 M³ Rochester-type dynamic flow chambers. Test atmospheres of TCE were generated by vaporization of the compound under different combinations of air flow- and temperature-controlled schedules. A heating mantle was placed around the dispersion flask to help controlling the degree of TCE vaporization, and a flow of nitrogen gas was introduced into the flask on the level of 2-3 inches above the liquid TCE surface. The nitrogen gas carried the gaseous TCE from a flask into the exposure chamber influent air stream through stainless steel tubing that was wrapped by a heating coil to prevent condensation of the gaseous TCE. The entire vapor generation system was enclosed in a plexiglass safety box under constant negative pressure. Exhaust air from the safety box and the inhalation chamber were vented through HEPA and activated charcoal filters prior to its release into the environment. The exposure chamber was operated at flow rates of 7 to 15 ft³ per minute (1/4 to 1/2 change of the chamber volume per min), and a negative pressure of 20 mm Hg was maintained at all times during operation of the chamber. The concentration of TCE in the exposure chamber was monitored by an on-line Miran 1B2 infrared spectrophotometer ($\lambda=10.9\mu\text{m}$) (Foxboro Co., Faxboro, MA) on the level of the animal cages. The Miran was calibrated with a closed loop system (Foxboro Co., Faxboro, MA) and the accuracy verified before each exposure session with liquid TCE injections that volatilized to produce concentrations spanning the calibration range.

Inhalation Exposure:

The inhalation exposure scheme and experiment design are presented in Table 1. Rats (5/group) were randomly assigned for either 2500 ppm repeated exposure, or 4500

ppm single exposure for . The repeated exposure was carried as one exposure session per day for three consecutive days, and the single exposure was finished with one session. All the inhalation exposure sessions lasted 120 min. In the kinetic study, the rats were sacrificed and samples were taken during and following the single 4500 ppm exposure. The repeated 2500 ppm exposure group were sacrificed and samples were taken during and following the last exposure. The sampling time points were 30, 60, and 120 min during and 0.5, 2, 4 hr following the exposures. In the GST study, the rats were exposed to either 2500 ppm one time a day for three days or single 4500 ppm exposure in the same manner as for the rats in the kinetic study. The exposed rats were housed with tap water and commercial rodent chow provided *ad libitum* for either 7 or 14 days before being sacrificed for sampling. Control animals were placed into the inhalation chamber operated without TCE for 120 min before sacrifice.

Tissue TCE Analysis:

The rats were terminated by cervical dislocation immediately upon their removal from the inhalation chamber, and their blood samples (1.0 ml) were withdrawn by closed chest cardiac puncture. Intact brains were quickly removed and dissected (<3 min) on a ice-chilled glass plate into medulla oblongata (MO), cerebellum (CE), cortex (CX), hypothalamus (HY), striatum (ST), midbrain (MB), and hippocampus (HC) according to Glowinsky and Iversen (1966). Samples from the lung, liver and kidney were also collected. The sampled tissue pieces were immediately capped in ice-chilled scintillation vials containing 4 ml of isoctane and 1 ml of 0.9% NaCl. The collected samples were processed and analyzed in the same day of exposures with the method described by Chen et al. (1993). The tissues were homogenized using an Ultra-Turrax SDT homogenizer (Tekmar, Cincinnati, OH) for approximately 10 seconds. The vials were then vortex-mixed for 30 sec and centrifuged at 1800 x g for 10 min at 4 °C in capped vials. Aliquots of the isoctane supernatant layer were transferred into 20 ml headspace vials, which were capped immediately with Teflon lined rubber septa and crimped to insure an airtight seal.

The vials were then placed into a Tekmar 7000/7050 headspace autosampler (Tekmar, Cincinnati, OH) that connected to a Shimadzu GC-14A gas chromatography (Shimadzu, Kyoto, Japan). Analyses were carried out using a stainless-steel column (182 cm x 0.317 cm) packed with 3% OV-17 (Alltech Associates, Deerfield, IL). The GC operating conditions were: headspace temperature, 60 °C; injection port temperature, 150 °C; column temperature, 70 °C; electron capture detector temperature, 260 °C. Each sample vial was equilibrated thermostatically for 5 min in the autosampler unit, pressurized for 30 sec with the carrier gas, and a fixed volume of the headspace injected automatically into the GC column. The limit of detection for TCE by the GC was less than 1 ng in 20 ml air.

Isolation and Assay of GSTs:

Brain and the extracerebral organ tissues were placed into scintillation vials and frozen immediately by placing the containers into acetone+dry ice mixture. The samples were subsequently kept frozen at -80 °C until use. The cytosolic fractions of the samples were prepared by ultracentrifugation (100,000 g) of tissues homogenized in 10 mM Tris/HCl, pH 7.8, containing 0.25 M sucrose, 10 mM EDTA, 2.0 mM EGTA and 2.0 mM dithiothreitol. All purification steps were carried out at 4 °C and cytosolic fractions were stored at -80 °C. Protein concentrations were determined by the method of Bradford (1976), with bovine γ -globulin as the standard. Cytosolic GST activity was measured by assaying the rate of conjugation of 1-chloro-2,4-dinitrobenzene (Habig and Jakoby, 1981). GSTs were isolated from cytosolic fractions by affinity chromatography as follows: equivalent amounts of cytosolic protein from each brain region were loaded on to an S-hexylglutathione-Sepharose affinity column and the material that did not bind was immediately loaded on to a GSH-Sepharose affinity column by the method of Hayes (1988). Both columns (0.7 cm x 1.0 cm) were equilibrated with 10 mM Tris/HCl, pH 7.8, containing 2.0 mM dithiothreitol (buffer A). The columns were then washed sequentially with buffer A, Buffer A containing 0.2 M NaCl (buffer B), and finally eluted with 2.0 ml of buffer B containing 5 mM S-hexylglutathione.

Separation of GST Subunit by HPLC:

A HPLC procedure utilizing a narrow-bore Vydac C18 30 nm reversed-phase column (2.1 mm × 25 cm) was used to separate the subunits of GSTs in the sample tissues. The high sensitivity of this procedure, requiring minimum tissue mass of 20-25 mg wet weight and with detection limit of any individual GST subunit at 0.2 pmol or approximately 50 ng of subunit protein, was necessary for the small mass of tissue from the various brain regions and the low levels of GST (0.2-0.4% of total cytosolic protein) presented. Solvent A was 0.1% trifluoroacetic acid in deionized water and solvent B was 70% acetonitrile/0.1% trifluoroacetic acid in deionized water. GST subunits were resolved by a 70 min linear gradient from 36.4 to 51.8% acetonitrile. The flow rate was 0.25 ml/min and u.v. absorbance at 214 and 280 nm was monitored. There was no evidence of memory between samples; recovery of GSTs ranged from 90 to 100%. GST subunits were previously identified by SDS/PAGE and by comparison with published HPLC profiles (Johnson et al., 1993). The amount of each GST subunit was determined from its peak height at 214 nm, by using the molar absorptivity (ϵ_{214}) for the individual subunits (Johnson et al., 1992). The concentrations of individual subunits were expressed as pmol of subunit protein/mg of cytosolic protein. Due to the variations in GST subunit 3 and 4, the measurement of these two subunits were pooled.

Statistical Analysis:

One-way analysis of variance (ANOVA), followed by a Duncan's multiple comparison procedure, was conducted to detect the differences in TCE brain tissue concentrations in different brain regions as well as the difference in GST subunits among the control and exposure groups. Independent t-test was used for analysis of paired sets of data. The significance level in all cases was set at $p<0.05$.

RESULTS

TCE Pharmacokinetics in Blood, Brain Regions and Extracerebral Organs:

The inhalation exposure scheme and the actual exposure concentrations of TCE are summarized in Table 1.

TCE uptake and elimination in blood during and following the inhalation exposures are shown in Figure 1. The TCE blood concentrations at both dose levels increased rapidly through the duration of the 120 min inhalation sessions, and they decreased immediately upon the termination of exposure until the last measurement at 4 hr post-exposure. The two TCE exposure regimes exhibited parallel overall pharmacokinetic behavior, and the differences was statistically significant at 120 min of exposure ($p \leq 0.01$) and 2 hr post exposure ($p \leq 0.05$). Blood had lower TCE concentration than any of the brain tissue samples at each time point measured at both dose levels, except for point of cortex in the higher dose group at 4 hr post exposure, which had about the same TCE concentration as the blood (Tables 2 & 3).

Significant differences in TCE concentrations were found in the different brain regions (Table 2). It is apparent that medulla oblongata, hypothalamus and striatum had relatively higher TCE concentrations, while tissues from the areas of cerebellum, cortex, hippocampus and midbrain had relatively lower TCE concentrations. The values in the various brain regions were uniformly lower in the low dose group than the high dose group. It is evident that the TCE brain regional concentration values in the higher dose group had larger variations than the lower dose, with the standard deviation ranging from 3.3 at post 4 hr to 89.1 at 120 min in the high dose, in contrast to 3.3 at post 4 hr and 35.2 at 60 min in the low dose (Table 2). In the brain measurements ranging from 30 min following the start of inhalation exposure to 4 hr after the termination of the exposure, there is a statistically significant difference in the TCE concentration among the brain regions. In most cases, the TCE concentrations were aggregated into two clusters, one composed of cortex, hippocampus and cerebellum, and the other usually included medulla

oblongata and striatum. Except for the 30 min measurement of the high dose group, the cortex region consistently had the lowest TCE concentration. Cortex TCE concentration ranged from 53-66% and 37-71% of the highest TCE concentrations in other brain regions at the corresponding time points, for the 2500 ppm and 4500 ppm exposure groups, respectively.

The values of area-under-the-concentration-time-curves (AUCs) of TCE in each brain regions were calculated by the trapezoidal rule using time points from 0 to 4 hr post exposure (Table 4). Since differences exist in the rate of TCE uptake in the different brain regions, it is possible that the C_{max} , represented by the TCE concentrations at 120 min in the current study, may not reflect accurately the total uptake and accumulations of the chemical in the brain regions. In fact, by comparing the rank order of TCE concentrations among the brain regions at 120 min and the rank order of their corresponding AUC values, one can find that the two pairs (of the high and low dose groups) of rank order do not match (Table 2 & 4). Among the instances are the incongruity of the AUC and C_{max} in hypothalamus at the low dose, and the inverted orders of hypothalamus and striatum in the two ranks at the high dose.

A simple pattern of rapid uptake phases and rapid elimination phase of TCE during and following inhalation exposure were also seen in the extracerebral organs (Table 3). TCE concentrations in the liver and kidney were invariably higher than in the blood, while the lung exhibited similar chemical levels as the blood during the elimination phase of the high dose group. The uptake of TCE was particularly rapid for lung and kidney at the high dose level, resulting in the highest concentrations at a single time points of any sampled tissue.

GST Subunits Distributions in Brain Regions:

The different subunits of GST clearly have widely varied levels of expression in the various brain regions (Table 5). Subunit 7 in most cases had the highest concentration, ranging approximately between 10 to 20 pmol/mg of cytosolic proteins, while subunit 8

protein levels were the lowest among all the brain regions, with only about a 20% of the amount of subunit 7. Protein levels of these subunits also varied widely among the brain regions. In no case was there a GST subunit that had a uniform distribution among the seven brain regions measured. Although they were in the same magnitudes, the values of each of the GST subunits were found to represent at least three statistically distinctive subsets of data. The overall variations of the data among the GST subunits, although statistically significant, were well in proportion in relations to the values in their respective subunit data sets. For instance, the standard deviation of subunit 8 levels was about 20% of that of subunit 7, indicating that variability in some cases between the subunits were similar among the brain regions. However, the rank orders of GST levels among these regions lacked apparent consistence when the subunits were compared to each other. For example the cerebellum had the highest level of subunit 6, but it also had the lowest levels of subunits 7 and 2.

The Effects of TCE Inhalation on GSTs in Brain Regions:

The effects of TCE inhalation on GST isozyme protein levels in the brain regions were relatively small and variable. In medulla oblongata (Figure 2), significantly higher levels of subunits 3&4 and 6 were detected in the high dose single exposure group at 14 days of post exposure (HD-14), but a decrease of enzyme levels was found with subunit 7 of the low dose group at 7 days post-exposure (LD-7) and 8 (HD-14) in cerebellum. While TCE inhalation did not produce any effects on the GSTs in hypothalamus, increases in subunit 7 of high dosage group at 7 days post-exposure (HD-7) and subunit 2 (HD-7), and decrease in subunit 8 from the low dose group at 14 days post-exposure (LD-14) were found in striatum (Figure 3). Significant increases of subunit 7 (HD-14), subunit 6 of both doses at 14 days, and subunit 11 of high dose at both time points were detected in midbrain (Figure 4). Decreases were found for subunit 6 (LD-7) in hippocampus (Figure 4) and subunit 8 in cortex for all four treatment groups (Figure 5). Although sporadic increases and decreases in GST subunit levels were detected by the statistical procedures,

in no instance was the change ever more than a doubling in activity (the largest was a 75.9% increase of subunit 6 from the control value in midbrain of the HD-14 group).

GSTs and the Effects of TCE Inhalation in Extracerebral Organs:

The GST subunit levels in the lung were very close to that in the brain, with subunits 6 and 11 particularly low (Table 6). The differences between the GST levels of liver and kidney (Table 6) and that of brain were dramatic, as the two organs had several times higher concentrations in some of their GST subunit levels compared to the brain, and there were also high levels of subunit 1 and 1', which were not detectable in the brain. It was also obvious that the liver and kidney had large differences in their relative compositions of GST isozymes, of which the subunits 3&4 were more than 10 times higher in the liver than in the kidney. The effects of TCE inhalation on the lung was negligible (Figure 6). In the liver, a suppression effect was seen for several subunits (Figure 6) at either a high dose treatment or a 14-day sacrificing group. This was in contrast to TCE effects on the kidney, in which enzyme induction was significant for several GST subunits. The increased levels of isozymes were mostly seen with the 7 day sacrifice groups.

GST Activity Assay and the Effects of TCE Inhalation:

The total GST activity, assayed for their conjugating capacity for 1-chloro-2,4-dinitrobenzene, are presented in Table 7. Differences existed in the control values of GST activity in the various brain regions, as hypothalamus had significantly higher values than all the other regions, followed by striatum and cerebellum. Cortex and medulla oblongata had the lowest values. Limited GST activity was found in the lung, which was even lower than that of brain tissues. Both liver and kidney had much higher GST activity than the brain, and among the two, the liver showed more than three times higher conjugating capacity than even the kidney. Inhalation exposure of TCE at 4500 ppm apparently had registered the most effect on the GST activities in brain regions of any measurement conducted. The 7- and 14-day groups at high dose as well as the 14-day group at low dose saw significant increases of GST activity in midbrain as well as in hippocampus; and

an increase in medulla oblongata was also evident at the high dose 14 day group. However, decreases of GST activity were found in cortex and striatum, both in the low dose groups. The effects of TCE exposure on the extracerebral organs also were not consistent either. While an increase was detected in the kidney, a decrease was found in the liver.

DISCUSSION

In the paradigm of administered dose—internal dose—response, the accurate assessment of dosimetry is seen as critical in making realistic predictions about the pharmacodynamic outcomes, which define the type and biological effects elicited by the chemical species that is (are) evolved from the pharmacokinetic process. The approach of using dose surrogates in the tissue (in contrast to the administered dose) as more toxicologically meaningful dosimeters have recently been used in developmental toxicology (Terry et al. 1994) and in chemical carcinogenesis (Heck, et al., 1996).

In the present study of TCE, the entity of the parent compound was used to describe the tissue dosimetry because the parent compound is believed to be primarily responsible for the CNS effects (Bruckner, et al., 1989). Chemical concentrations in brain tissue have been previously used to correlate neurotoxicity with toxicokinetics of VOCs. Bruckner and Peterson (1981) reported that toluene levels in blood and whole brain had high predictive value in relation to its effects on CNS depression. However, an investigation on the regional brain distribution of toluene have revealed significant difference in the toluene concentrations in various brain regions (Gospe and Calaban, 1988). For instance, the highest toluene concentrations in medulla oblongata were upto 2.7 fold higher than the concentrations in other brain regions following inhalation exposure. A similar situation has been reported for 1,1,1-trichloroethane (TRI) neurotoxicity. Warren et al. (1993) found a good degree of correlation between rat brain concentration of TRI and its depressant effects on operant behavioral response. However, the chemical concentration

was measured with tissue homogenate of whole brain, and it has subsequently been found that TRI, too, has a substantially varied uptake and distribution among the various anatomical regions in rat brain (You et al., 1995).

The current study has confirmed that TCE also has a differential uptake and distribution among the brain regions. The basic pattern of rapid TCE uptake and distribution are similar at both dosage levels in all the tissues measured, including brain regions and the extracerebral organs. The fastest increase in the absorption and distribution of TCE was seen in the first 30 min of the exposure, during which the concentration in medulla oblongata was more than twice that of the blood TCE concentration. Similarly large disparities were also found between blood TCE concentration and other brain regions and throughout the exposure phase. Among the brain regions, medulla oblongata and striatum showed higher concentrations than cortex, cerebellum, and hippocampus. The largest overall difference of TCE concentrations between two regions was found with cortex and medulla oblongata, in which the AUCs of cortex accounted for 61 and 54% of the AUCs of medulla oblongata in the low and high dosage groups, respectively. One might expect that lipid content of the brain regions would account for the regional brain differences in the toxicokinetics of this highly lipophilic chemical. However, the lack of total consistence in the two rank orders of TCE tissue concentration AUCs at different dosage levels suggests that more factors other than the lipid content of the brain regions (Gospe and Calaban, 1989) must play a role in determining the distribution of TCE into the tissues.

While our understanding is still limited on the effects exerted by TCE on the cellular and molecular levels in relation to its neurotoxicity, a lot has been learned about the metabolism of TCE (Bruckner et al., 1989; Goeptar et al., 1995). The principle site of TCE metabolism is the liver where oxidative metabolism takes place via the actions of cytochrome P-450-dependent monooxygenases, principally the isozyme P-450 IIE1 (Raucy et al., 1993; Geoptar et al., 1995). A substantial portion of the terminal products

of the dose-dependent metabolism of TCE is trichloroethanol, mostly in the form of glucuronide conjugation that is readily excreted in the urine (Arlien-Søbory, 1992; Templin et al., 1995). A minor metabolic pathway for TCE, however, involves glutathione conjugation, which is catalyzed by GSTs and represents the first step in the mercapturic acid pathway (Bruckner et al., 1989; Dekant et al., 1990; Goepfert et al., 1995). It needs to be point out, however, that it does not require a GST substrate to exert effects on the expression of GSTs. This was demonstrated with lead for instance, which was not a GST substrate but was nonetheless capable of increasing markedly a number of GST isoforms in adult and developing rats (Moser et al., 1995). TCE has also been reported to have an effect on other drug metabolism enzymes, including cytochrome P-450, cytochrome *c* reductase, glucuronyl transferase, and hexobarbital hydroxylase (Pessayre et al., 1979).

It is well documented that various GST isoforms can be induced by an array of pathological and toxicological factors including cellular malignancy and exposure to various drugs and chemical agents (Boyer, 1989). However, two factors complicate the interpretations of most research data on this subject. Large variations exist in the expression levels of GST isoforms among different species, strains, organ and tissue types, and developmental stages (Boyer, 1989; Mannervik and Danielson, 1988; Ketterer et al., 1988). Secondly, the susceptibilities of different isoforms to inducing agents are very different (Ketterer et al., 1988; Boyer, 1989).

A major contribution of the present study was the definitive characterization of the variations of GST isoforms in the brain regions at the control level. The GST protein levels in all the brain regions were rather low compared to the liver and kidney, an indication that the metabolic potential of the brain is limited. The results of TCE exposure suggest mixed effects on the GST isoforms, as most of the brain regions and organs showed non-systematic changes of subunit protein levels. A few induction effects have been detected upon TCE inhalation, for example with subunit 7, 6, and 11 in midbrain and

with subunit 3&4 and 6 in striatum. There were also decreases in some other instances. These results are in agreement with a previous report of TCE effects on metabolic enzymes (Pessayre et al., 1979), in which it decreased the levels of some enzymes (cytochrome P-450, ethyl-morphine demethylase, and hexobarbital hydroxylase) but increased others (NADPH-cytochrome *c* reductase and aniline hydroxylase). TCE metabolites, including a possible epoxide, chloral hydrate, trichloroethanol and trichloroacetate (Arlien-Søborg, 1992; Davidson and Beliles, 1991) may have different effects towards the regulations of GST isoforms. It is known that cytochrome P-450 IIE1, the major first phase metabolic enzyme for TCE, also has a differential distribution among the brain regions (Warner and Gustafsson, 1993; Anandatheerthavarada et al., 1993), suggesting that the degree of metabolism and proportionality of the metabolites of TCE are not uniform among the brain regions as well. The complexity of TCE metabolism, plus the fact that the GSTs are products of a multi-gene family (Boyer, 1989), make it very difficult to correlate tissue dosage of a chemical with its potential effects on the GST isozymes. It is likely that these changes, as seen in the GST subunits protein levels and the total GST activities, while statistically significant, represent limited biological and toxicological significance. The relatively small magnitudes of those effects would infer that TCE effects on GST isozymes in Sprague-Dawley rats are nonsystematic and bear little direct correlation with the tissue dose of the parent compound. However, investigations are warranted to probe possible contributions of the metabolites to the regulations of GSTs.

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